TECHNICAL REPORT

ISO TR 10993-9

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Biological evaluation of medical devices —

Part 9:
Degradation of materials related to biological testing

Évaluation biologique des dispositifs médicaux —
Partie 9: Dégradation des matériaux relative à l'évaluation biologique

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Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The main task of technical committees is to prepare International Standards, but in exceptional circumstances a technical committee may propose the publication of a Technical Report of one of the following types:

— type 1, when the required support cannot be obtained for the publication of an International Standard, despite repeated efforts;

— type 2, when the subject is still under technical development or where for any other reason there is the future but not immediate possibility of an agreement on an International Standard;

— type 3, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example).

Technical Reports of types 1 and 2 are subject to review within three years of publication, to decide whether they can be transformed into International Standards. Technical Reports of type 3 do not necessarily have to be reviewed until the data they provide are considered to be no longer valid or useful.

ISO/TR 10993-9, which is a Technical Report of type 2, was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This document is being issued in the type 2 Technical Report series of publications (according to subclause G.4.2.2 of part 1 of the ISO/IEC Directives, 1992) as a "prospective standard for provisional application" in the field of degradation of materials because there is an urgent need for guidance on how standards in this field should be used to meet an identified need.

This document is not to be regarded as an "International Standard". It is proposed for provisional application so that information and experience of its use in practice may be gathered. Comments on the content of this document should be sent to the ISO Central Secretariat.

A review of this type 2 Technical Report will be carried out not later than two years after its publication with the options of: extension for another two years; conversion into an International Standard; or withdrawal.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Guidance on selection of tests*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for cytotoxicity: in vitro methods*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Degradation of materials related to biological testing* [Technical Report]

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymers*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from coated and uncoated metals and alloys*

— *Part 16: General guidance on toxicokinetic study design for degradation products and leachables from medical devices*

— *Part 17: Glutaraldehyde and formaldehyde residues in industrially sterilized medical devices*

Annexes A, B and C of this part of ISO 10993 are for information only.

Introduction

Attention is drawn to the definition of "medical device" (see 3.1).

When the material of an implanted device experiences a decrease in its mechanical properties and/or mass, it is referred to as degradation for polymers and ceramics, and corrosion for metals and alloys. For those medical devices and materials which experience repeated stress cycles during use, the biological environment may reduce their anticipated fatigue life or endurance limit.

The stability of materials and devices in the biological environment is influenced by the conditions of the service environment as well as the chemical composition, additives, processing aides, impurities, manufacturing processes and decontamination/sterilization.

In addition to the principal components, most polymers contain additives such as antioxidants and stabilizers and all materials contain either minor components or impurities which may be leachable and enter the biological environment and induce a tissue response even if the principal component is stable. Most devices rely upon their stability to ensure safe and efficacious performance. Others, such as resorbable sutures, are designed intentionally to degrade.

While the ability to predict the stability of materials and medical devices *in vivo* is of great importance, the extrapolation of *in vitro* data to the clinical service environment remains a difficult, yet-to-be resolved problem. *In vitro* methods for assessing degradation of materials and medical devices range from the very simple, e.g. exposure to 0,9 % isotonic saline solution, to more complex exposure to solutions containing enzymes, phospholipids, etc. In addition, the material/device can be subjected to external mechanical stress/load during testing. A sequential approach to *in vitro* degradation testing of medical devices and materials involving both accelerated and real-time testing techniques is shown in figure 0.1.

Selection of methods for accelerating degradation testing in a manner which is representative of the service environment remains the responsibility of the designers of the *in vitro* test schemes used. Currently, there are no standard practices, methods or guidelines for the assessment of the degradation of medical devices and materials, and of the degradation products formed, from degradation of these materials and devices in the biological environment. There are, however, methods used for extracting leachable fractions from materials and devices to be used in biological response testing (see, for example, ISO 10993-3, ISO 10993-5, ISO 10993-6 and ISO 10993-7).

The extraction media used may be selected to be compatible with the biological test and may not necessarily represent an adequate extraction of the material. There is a need for additional research to develop accelerated methods for evaluating materials degradation in the human body.

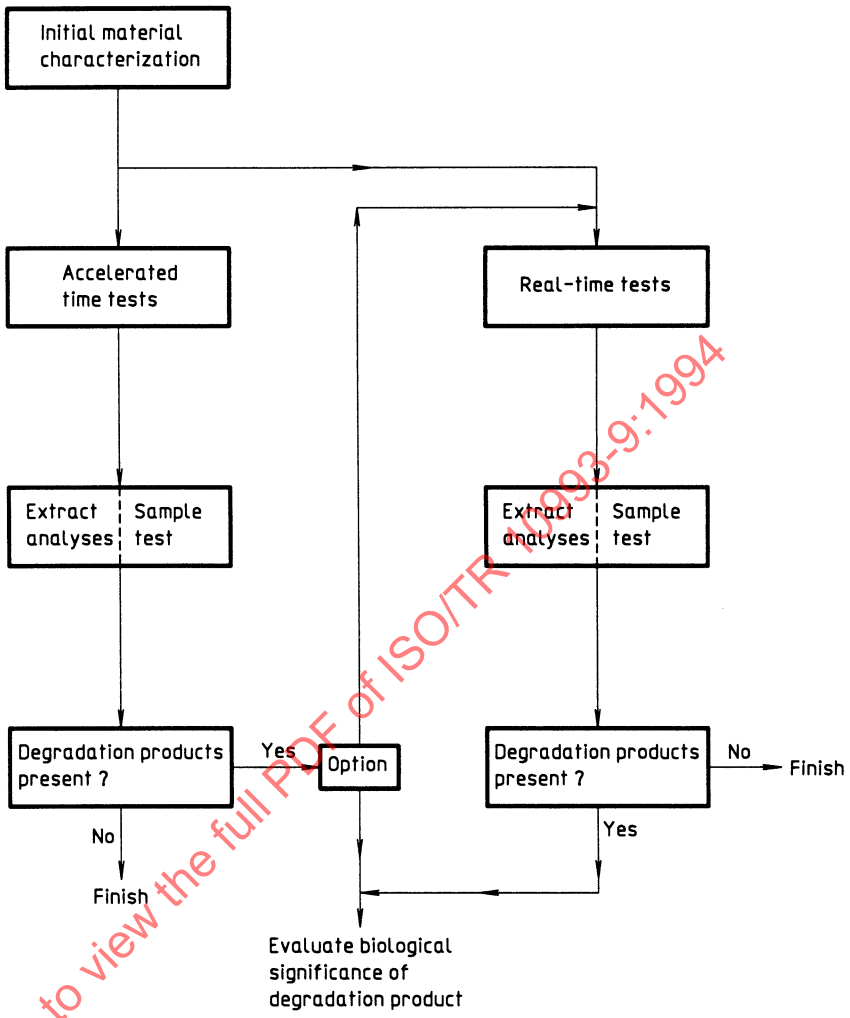

Figure 0.1 — Degradation product evaluation scheme

Biological evaluation of medical devices —

Part 9:
Degradation of materials related to biological testing

1 Scope

This Technical Report aims to facilitate the design of test procedures which are used to evaluate the biological responses to degradation products released from medical devices. Following brief descriptions of each major class of material used for medical devices, this Technical Report focuses on the likely mechanisms of degradation of these materials when they are used in a biological service environment. Accelerated time and real-time degradation testing environments for devices and materials are suggested along with characterization techniques for the degradation products. Finally, an approach for identifying and quantifying degradation products obtained from explanted devices and tissues is proposed.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Technical Report. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this Technical Report are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-3:1992, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.*

ISO 10993-5:1992, *Biological evaluation of medical devices — Part 5: Tests for cytotoxicity: in vitro methods.*

ISO 10993-6:1994, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation.*

ISO 10993-7:—[1], *Biological evaluation of medical devices — Part 7: Ethylene oxide sterilization residuals.*

ISO 10993-12:—[1], *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials.*

3 Definitions

For the purposes of this Technical Report, the definitions in ISO 10993-1, ISO 10993-3 and 10993-5, and the following definitions apply.

3.1 medical device: Any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, intended by the manufacturer to be used for human beings solely or principally for the purposes of:

— diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or handicap;

— investigation, replacement or modification of the anatomy or of a physiological process;

1) To be published.

— control of conception;

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means. (Repeated for convenience from ISO 10993-1:1992, definition 3.1.)

3.2 accelerated time test: Laboratory test designed to speed up the degradation of a material/device which would normally take place in the service environment.

3.3 aluminium oxide ceramic: High density, high purity Al_2O_3 which may be a fine-grained polycrystalline, semicrystalline, amorphous or single crystal material.

3.4 amorphous polymer: Macromolecules which do not form crystalline structures.

3.5 antistatic agent: Additive which increases surface conductivity of the material and thus reduces the buildup of charge on the surface.

3.6 bioabsorption: Dynamic process involving the interaction of the body with a biomaterial which results in the breakdown and dissolution or uptake of the material in the physiological environment.

3.7 bioadsorption: Deposition of components of tissue and body fluids on the surface of a material/device.

3.8 bioceramic: Ceramic which upon implantation is transformed into less soluble minerals.

3.9 biodegradation: Alteration undergone by the biomaterial or medical device involving loss of their integrity or performance when exposed to a physiological or simulated environment.

3.10 bioerosion: Dissolution or fragmentation of a biomaterial implanted in the body occurring as a result of surface reactions.

3.11 bioglass; bioglass ceramic: Glass based on SiO_2 which has a specific surface reactivity that enhances the interaction with surrounding tissue due to the action of additives, primarily alkali oxides.

3.12 biomaterial: Synthetic, natural or modified natural material intended to be in contact and interact with the biological system.

3.13 bioresorption: Process by which biomaterials are degraded in the physiological environment and the byproducts eliminated or completely bioabsorbed.

3.14 biostability: Quality of a biomaterial or a medical device of which the physical, chemical and mechanical or other changes are not modified with respect to its behaviour, function or performance within the biological environment.

3.15 biotransformation: Any change which a biomaterial undergoes in the body due to the interaction between the material and the physiological constituents.

3.16 branched polymer: Long-chain molecules with side chains attached to the backbone of the polymer. Often soluble in the same solvents as the corresponding linear polymers.

3.17 calcium phosphate ceramic: Ceramic based on calcium and phosphorus oxide containing one or more calcium phosphate phases.

3.18 coating: Deposited layer or covering on a biomaterial or medical/dental device which is intended to protect or enhance the performance of the device or biomaterial.

3.19 [random] [regular] [block] [graft] copolymer: Polymer consisting of different repeating units (made from two or more different monomers), having different degrees of order.

3.20 corrosion: Chemical reaction of a solid material (usually a metal or alloy) with the environment which causes measurable material property changes.

3.21 crevice corrosion: Usually accelerated chemical reaction in a small fissure, pit or crack of a metal or alloy within a corrosive environment.

3.22 crosslinked [network] polymer: Polymer with chemical linkages between the chains.

3.23 degradation product: Byproduct of a material which is generated by the breakdown or decomposition of the material.

3.24 depolymerization: Reversion of a polymer to its monomer(s) or to a polymer of lower relative molecular mass.

3.25 deposit: Layer or covering laid down on a biomaterial or medical device.

3.26 elastomer: Polymer which maintains its elastic properties in the temperature range between glass

transition temperature and the temperature at which the elastomer begins to flow.

3.27 electrolyte: Substance, usually in solution, which will transmit an electric current by ions.

3.28 environmental stress-cracking: Cracking of stressed materials subjected to certain conditions: the stress may be externally applied, or internally introduced during processing.

3.29 enzymatic degradation: Degradation of materials enhanced and/or initiated by enzymes.

3.30 extraction medium: Liquid which does not dissolve a material or device, but does induce the release of one or more extractable components.

3.31 fatigue corrosion: Combined effect of dynamic loads and chemical attack on metal or alloy properties within a corrosive environment.

3.32 fretting corrosion: Combined interaction of wear and chemical attack on a metal or alloy within a corrosive environment.

3.33 fibre: Arrangement of molecules into a long tubular shape where the length is of the order of 100 times the diameter. Fibre can be continuous or specific lengths.

3.34 filler: Various forms of inorganic or organic materials added to alter material properties.

3.35 final product: Medical device in its "as-used" state.

3.36 homopolymer: Polymer consisting of identical repeating units (made from a single monomer).

3.37 hydrolysis: Chemical bond-cleavage which involves attack by neutral or ionized water in acidic, neutral or basic aqueous media.

3.38 implant coating interface: Zone between the substrate and the coating.

3.39 implant tissue interface: Zone between the implant material and the tissue.

3.40 inert bioceramic: Bioceramic which undergoes no measurable chemical change during long-term contact with the biological environment.

3.41 interface: Zone of contact between two surfaces.

3.42 *in vitro* degradation: Changes in a material exposed to an environment simulating *in vivo* conditions.

3.43 *in vivo* degradation: Structural, physical, mechanical and chemical changes in a material that are induced by the organism.

3.44 leachable: Extractable component, such as additives, monomers and low relative molecular mass constituents in polymeric materials.

3.45 linear polymer: Long chains of uncrosslinked backbone atoms which form a macromolecule.

3.46 lubricant: Substance added to enhance material flow and handling characteristics (e.g. during injection-moulding, extrusion, etc.).

3.47 matrix: Embedding material for fibres or fillers.

3.48 monomer: Any molecule that can be converted into a polymer.

3.49 natural polymer: Macromolecules of natural origin, such as collagen, cellulose, polyaminoacids, rubber, etc.

3.50 oxidative degradation: Reaction occurring due to air (oxygen) or oxidizing agents leading to chain cleavage of chemical bonds.

3.51 pigment: Insoluble colorant added to a material.

3.52 plasticizer: Substance added to polymers which modifies their forming properties and increases the flexibility of products made from these polymers.

3.53 plastics: Synthetic polymers containing additives which enhance polymer processing and/or the final properties of the resulting products.

3.54 polymer blend: Two or more polymers mixed together or in solution to form a product which has properties very often different from those of individual polymers.

3.55 potentiostatic polarization method: Method equal to chronoamperometry with a constant potential: the time-dependent measurement of the current density going through a material/solution interface at a definite potential.

3.56 potentiodynamic polarization method: Method equal to voltamperometry with a linear variation of the potential: the potential-dependent

measurements of the current densities going through a material/solution interphase.

3.57 real-time test: Laboratory test performed to assess degradation of devices or materials which does not employ accelerating factors such as temperature, rapid cycling, etc.

3.58 resorbable bioceramic: Ceramic which upon implantation is dissolved into the surrounding body fluids and tissues.

3.59 resorbable cement: Cement formed from a mixture of inorganic powders, which sets upon mixing with water or dilute acidic or basic solutions and when implanted is dissolved into the surrounding body fluids and tissues.

3.60 resorbable polymer: Polymer deliberately designed to be biotransformed *in vivo* to a non-harmful byproduct which is subsequently eliminated from the organism.

3.61 semicrystalline polymer: Macromolecules able to crystallize partially from the melt or solution.

3.62 solvent: Substance which causes a gas, solid or liquid to dissolve without altering its (chemical)/(molecular) identity.

3.63 stabilizer: Additive added to materials to prevent chemical reactions.

3.64 stable polymer: Polymer which does not undergo any measurable changes when exposed to its service environment.

3.65 stress corrosion: Combined effect of static and/or dynamic stress and chemical attack on metal or alloy properties within a corrosive environment.

3.66 substrate: Material covered by a coating.

3.67 surface-reactive bioceramic: Ceramic which upon implantation shows a surface reaction which may lead to formation of chemical bonding at the implant/tissue interface.

3.68 surgical implant: Medical device implanted in the body by surgical means.

3.69 synthetic polymer: Man-made polymer.

3.70 thermoplastics: Polymers capable of undergoing repeated flow and solidification upon heating/cooling.

3.71 thermoset: Polymer that exists initially as a reactive compound, which upon heating, undergoes a reaction to form a solid, highly crosslinked insoluble matrix.

4 Classification of materials

Biomedical devices are constructed from a wide variety of materials, representing each of the major material classes. These classes include polymers, metals and alloys, ceramics, composites and coatings. A brief introduction to each class as related to its application as a biomedical material is given below.

4.1 Polymers

Polymers are long-chain molecules composed of small, simple repeating units called monomers. The repeating unit is usually equivalent, or nearly equivalent, to the monomer from which the polymer was obtained. Frequently, polymeric materials with relative molecular masses of approximately 20 000, and/or consisting of less than 1 000 to 2 000 repeating units in the chain, are called low molecular mass polymers, while polymeric materials above 20 000 and/or consisting of more than 2 000 repeating units in the chain are called high molecular mass polymers. The term polymer usually refers to the pure material which results from the polymerization process.

Depending on the structure of the polymer chain, polymers are called homopolymers, copolymers, or linear, branched or crosslinked polymers. Another class of polymeric materials is polymer blends consisting of a physical mixture of two or more polymers. Furthermore, depending on the material properties and behaviour, polymers can be divided into thermoplastics or thermosets. In relation to their origin, polymers can be further categorized as natural or synthetic.

4.2 Ceramics

Ceramics are inorganic materials prepared by high temperature thermal processes which form ionic bonds.

The use of ceramics as materials for medical devices originated from research in the early 1970's, which led to the introduction of a variety of bioceramics such as aluminium oxide, glass ceramics and calcium phosphate ceramics. This discussion mainly focuses on four types of ceramics, namely, aluminium oxide, glass ceramics, calcium phosphate ceramics and carbons. These types exhibit three different material responses, i.e. minimally reactive (aluminium oxide), surface reactive (glass ceramics) and resorbable

(calcium phosphate ceramics). The classification of calcium phosphate as a resorbable material is, at present, controversial since the degradation rate of the different types of calcium phosphate varies with changes in its stoichiometry and crystal structure. Wide differences of opinion exist on degradation of hydroxyapatite, a calcium phosphate ceramic, with some investigators classifying it as resorbable, while others consider it nonresorbable. A brief description of each of the major classes of ceramic materials is provided below.

4.2.1 Aluminium oxide is used in high purity form. Devices are, usually, fabricated from fine-grained polycrystalline material and used in load-bearing applications because of its high compressive strength, flexural strength and fatigue resistance. Both strength and fatigue resistance may decrease when it is exposed to physiological fluids.

4.2.2 Glass ceramics can be produced in various compositions. The basis of these ceramics is SiO_2 and P_2O_5. Depending on the type, CaF_2, K_2O, MgO, Al_2O_3, TiO_2 and B_2O_3 can also be found. The surface reactivity is dependent on the composition of the material. For some compositions, hydroxyapatite crystals are expected to nucleate at the implant surface, causing a firm bond with surrounding collagen and bone.

4.2.3 The calcium phosphate ceramics, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, tricalcium phosphate $Ca_3(PO_4)_2$, and a biphasic combination of these two crystal structures are currently commercially available for use on surgical implants. Other calcium phosphates are currently being investigated for application as biomaterials as well; for example, fluorapatite, magnesium whitlockite and tetracalcium phosphate. The starting material for most hydroxyapatite ceramics is a powder with hydroxyapatite lattice, whereas hydrothermally processed coral materials such as porites are also used.

4.2.4 Ceramics can be fabricated in dense and porous forms. Dense ceramics are used as blocks, granules or coatings. An increase in porosity decreases compressive strength and increases surface area and rate of dissolution. High sintering temperatures tend to reduce the microporosity (pore size less than 3 µm) of ceramics and may relate to the controversy surrounding the degradability of hydroxyapatites, the non-degradable form approaching theoretical density.

NOTE 1 A more complete listing of specific ceramic materials used medically is provided in table 1.

Table 1 — Classification of ceramic materials

Class	References	Form and composition
Ceramic based on alumina	ISO 6474	Dense sintered crystalline alumina (A1203)
	—	99,9 % pure porous alumina
	—	97 % pure porous alumina
	—	Zirconia reinforced alumina
	—	Calcium phosphate alumina
	—	Single crystal of alumina
Ceramic based on silicate glass	—	Porous vitroceramic
	—	Dense vitroceramic
Ceramic based on calcium phosphate	—	Dense sintered hydroxyapatite
	—	Porous sintered hydroxyapatite
	—	Dense sintered beta TCP
	—	Porous sintered beta TCP
	—	Other types
Ceramic based on calcium carbonate	—	Porous sintered calcium carbonate
	—	Natural porous calcium carbonate
Ceramic based on zirconia	—	Alumina stabilized zirconia
	—	Yttria partially stabilized zirconia
	—	Stabilized zirconia
	—	Ceria partially stabilized zirconia
Dental ceramic based on feldspar	ISO 6872 ISO 9693	Reinforced with leucite
	—	Reinforced with alumina
	—	Reinforced with mica

4.3 Carbons

Carbons include graphite, diamond, and vitreous and pyrolytic carbons. They can be prepared in high purity and are generally considered stable in the physiological environment. Examples of the different carbon forms used as medical non-reactive materials follow.

4.3.1 Pyrolytic carbon: low temperature isotropic and high temperature isotropic carbon materials have a laminar, isotropic, granular or columnar structure and may be pure carbon or alloyed with various carbides, usually silicon. The structure is generally described as turbostratic and consists of poorly defined crystallites.

4.3.2 Glassy or polymeric carbons: obtained from the thermal pyrolysis (approximately 1 000 °C) of selected polymers and may be monolithic, porous or reticulated.

4.3.3 Artificial graphites: produced from a variety of starting materials such as petroleum or naturally occurring cokes and yield bulk structures of varying grain size, crystallite orientation, purity, porosity, strength and particle size.

4.3.4 Carbon fibres: formed from spun polymeric fibres which are subsequently pyrolysed to yield structures of unusual strength and stiffness. The properties are a function of polymer precursor and processing history. More recently, carbon fibres have been grown from the vapour phase.

4.3.5 Charcoal: perhaps the oldest and most diverse materials with interesting absorptive properties are produced from many organic materials spanning the range from wood to coconut shells to animal bones.

4.4 Composites

4.4.1 Composites consist of at least two different types of materials (fibres or fillers, and matrix) or of one material with different structures (self-reinforced material). The principle of improved mechanical properties is based on the interaction of fibre and/or fillers with the matrix. The direct contribution of matrix to the mechanical properties of the composite is relatively small. The function of matrix is to transfer load from matrix to fibres over the fibre/matrix interface, to protect the fibres and to shape the device. For the fibre and matrix to peform effectively, good fibre/matrix adhesion is required.

A sufficient strength over the predicted time of application is ensured only if there is good adhesion between fibre and matrix over the whole implantation period. Agents on the fibre surface, for example textile finishes, coatings, sizing or lubricants can interfere with the contact integrity, so that the strength is reduced. Therefore, fibres for reinforcement shall be cleaned, or special surface treatment be used.

4.4.2 Mechanical properties of composites are highly correlated with the specific materials used, and the quantity and orientation of the fibres and/or filler. The continuous fibre-reinforced materials can be oriented to achieve different degrees and patterns of anisotropy. In general, the reinforcing material can be fibrous, powdered, spherical, crystalline or whiskered and organic, inorganic, metallic or ceramic material. The fibre materials used today are made of carbon, glass, aramid, ceramic, and resorbable materials such as polyglycolide (PGA), polylactide (PLA) and others.

4.4.3 Typical matrices in the medical field are shown in table 2.

Reinforced polymeric materials may have mechanical properties comparable to metals in reference to strength but with reduced mass.

4.4.4 Fillers can be of organic or of inorganic origin with a particle size ranging from micrometres to one millimetre. They can consist of a generally different class of materials from the matrix material, or of the same material. The fillers can be composites themselves, or may be carriers of drugs. The aim of filling a matrix material is to increase the strength, to reduce shrinkage and the thermal coefficient of expansion, to improve the handling properties, or, finally, to achieve radio-opacity, etc. For fillers, especially in dental applications, silica glass, metal oxides and acrylates are in use.

Composites for biomedical applications usually use epoxy resins, thermoplastics such as polysulfone, polycarbonate, polylactide or polyglycolide and inorganic materials such as ceramics as matrix. In most cases, the polymeric matrix material is amorphous or semicrystalline and the mechanical properties are relatively weak compared to the reinforcing fillers.

4.5 Metals and alloys

4.5.1 Metals are opaque, crystalline, chemically homogeneous substances which are conductors of heat and electricity. Most elemental metals are malleable and ductile; thus they can be plastically deformed within certain limits without breaking.

Metals are classified in a number of ways according either to their use or to their various properties. In industry, they are frequently classified as ferrous and non-ferrous metals, the former being primarily iron and the latter virtually all others. On a chemical basis, differentiation may be made between the noble metals, e.g. gold and platinum, and the active metals such as sodium and magnesium.

Metals may readily dissolve other metallic and some non-metallic elements, forming alloys with very specific properties which may differ substantially from those of the major alloy constituent. An alloy is normally made by melting the constituents together, or by sintering a compacted powder mixture of the alloy components (solid state diffusion). They can also be formed by electrodeposition from an electrolyte which contains the alloy constituents in the form of

Table 2 — Materials for composites

Matrix	Reinforcement	
	Fibres	Fillers
Resins Epoxy Polyacrylates Polymethacrylates Polyesters Silicones Polyurethanes **Thermoplastics** Polyolefins Polycarbonates Polysulfones Polyetherketones Polyesters **Resorbable polymers** Polyglycolide and its copolymers Polylactide and its copolymers Polydioxanone Polyhydroxybutyrate Natural origin **Others** Carbons **Ceramics** Hydroxyapatite Glass ceramics Calcium carbonate ceramics Calcium phosphate ceramics	**Polymeric** Polyesters Polyolefins Polyetherketones Polyurethanes **Resorbable polymers** Polyglycolide and its copolymers Polylactide and its copolymers Polydioxanone Aromatic polyamides **Others** Carbon Glass Resorbable glasses **Ceramics** Hydroxyapatite Tricalcium phosphate	**Organic** Polyacrylate Polymethacrylate Composite organic/inorganic **Inorganic** Silicon dioxide Silanes Glass Ytterbium trifluoride Yttrium fluoride Other metaloxides Hydroxyapatite Tricalcium phosphate

salts. An alloy in the solid state is also crystalline. Some properties of an alloy depend upon the structure whereas others depend on arrangements of the metal atoms within the crystal lattice. The former are called structure-sensitive properties and the latter structure-insensitive properties.

4.6 Coatings

Coatings are deposited layers of covering materials applied to a medical device for the purpose of protecting and/or enhancing the device performance.

The composition and form of the coating on a surgical implant is related to the function of the device and/or the manufacturing process used to produce the device. These functions and processes can be classified in the following manner.

4.6.1 Classification according to function

— mechanical function: sliding and wear resistance, shear-delamination resistance, fracture resistance, etc.

— chemical function: corrosion/erosion resistance, oxidation-reduction, chemical reaction, etc.

— tissue integration function: mechanical or biological anchoring in the biological environment

4.6.2 Classification according to manufacturing process

— chemical vapour or liquid deposition

— electrodeposition

— physical vapour deposition

— sputtering

— ion beam deposition

— plasma deposition

— spraying deposition

— enamelling

— sintering

— electron beam deposition

— dip coating

— others

NOTE 2 A further classification of coatings by generic class of material is provided in the following, and in table 3. Reference made to titanium in the tables implies both commercially pure and alloyed titanium.

4.6.3 Metal coatings

— titanium and its alloys

— tantalum

— platinum

— gold

4.6.4 Ceramic coatings

— oxides

— glass

— carbon

— carbide

— nitride

— calcium phosphates

4.6.5 Polymer coatings

— synthetic silicone

— polytetrafluoroethylene

— polysulfone

— polyethylene

— polyester

— polyparaxylylenes

— epoxy resins

— polyurethanes

— others

5 Materials degradation and related mechanisms

5.1 General

Materials exposed to the body environment undergo changes (hydrolysis, corrosion, etc.), as a result of chemical, physical, mechanical and biological interactions between the active living environment and the material. Advanced stages of degradation of medical materials and devices often result in a change in their properties and, hence, their functionality as well as their biological response.

The living body is an aggressive environment for most types of material or medical devices it contacts, and may cause material degradation. In many cases determination of the exact mechanisms of *in vivo* degradation of materials remains an unsolved problem. In others, *in vivo* degradation is known to be due to chemical hydrolysis or oxidation possibly enhanced by enzymes or other biological agents. Factors such as digestion of material fragments by macrophages and giant cells, bacterial activity, action of lipids, trace elements, salts, etc. may play a role in this process.

Products of degradation and/or released constituents may be eliminated from or accumulated by the body. This can lead to adverse local or systemic effects. These effects may be mediated by chemical, physical or biological phenomena.

The effect of infection on the degradation of biomaterials is not well understood. It may be related to the inflammatory reaction which occurs with infection or to direct interaction between microorganisms and the biomaterial.

Table 3 — Coating materials

Class	Form and composition	Observations
Titanium coating	Titanium plasma sprayed coating	On to titanium substrate
	Titanium coating	On to metallic substrate
Carbon coating	Pyrolitic carbon coating by CVD or CVI process	On to carbon fibre substrate
	Ion beam deposited carbon	For thin coating on to any material
Calcium phosphate coating	Calcium phosphates deposited by chemical liquid spray process (CLD)	For thin coating on to porous material
	Calcium phosphate sintered coating	
	Plasma sprayed calcium phosphate	On to titanium and cobalt chromium alloy substrate
	Sputtered calcium phosphate	Usually on to titanium substrate
	Calcium phosphate deposited by electrolytic process	On to titanium substrate
Alumina coating	Sputtered crystalline aluminium oxide	With metallic substrate
	Amorphous deposited aluminium oxide	With metallic substrate
	Porous alumina	With titanium substrate
	Porous coating 99,9 % pure alumina	With titanium and tantalum substrate
	Porous coating 97 % pure alumina	With titanium and tantalum substrate
	Cermet (A1203 + Ti)	—
Glass coating	Silica based ceramic coating	Fused on to alloy substrate

5.2 Polymers

5.2.1 The degradation of synthetic polymers in a biological environment is a complex process. The degradation generally proceeds via a surface-erosion process and/or a process involving the internal bulk of the material.

5.2.2 The surface-erosion process is characterized by the following steps:

a) adsorption of the liquids to the surface of the polymer;

b) diffusion of the liquids into the thin surface layer of the material, usually resulting in disruption of secondary bonds;

c) chemical reaction between the liquids and covalent bonds of the polymer leading to chain cleavage, which is manifested by a decrease in material strength and fragmentation;

d) diffusion of degradation products towards the surface of the material;

e) desorption (dissolution) of the degradation products from the surface of the material and phagocytosis of the smaller fragments.

5.2.3 The internal (bulk) process usually proceeds as follows:

a) adsorption of the liquids to the surface of the polymer;

b) diffusion of the liquids into the bulk (volume) of the polymer (both processes are accompanied by disruption of the van der Waals forces and eventually hydrogen bonding);

c) chemical reaction between the liquids and unstable covalent bonds of the polymer leading to chain cleavage, which is manifested by a decrease in material strength and fragmentation;

d) diffusion of degradation products towards the surface of the material;

e) desorption (dissolution) of the degradation products from the surface of the material and phagocytosis of the smaller fragments.

Thus, it seems evident that the rate of sorption of body liquids into a polymeric material determines to a great extent its molecular stability *in vivo*, while the time the material spends in contact with the environment affects the extent of its degradation. In the case of natural polymers where enzyme-catalysed reactions play a major role in the degradation, it is assumed that the reaction proceeds by the following two steps: first, an enzyme-substrate complex is formed, followed by decomposition of the complex into reaction products with regeneration of the enzyme.

It has been observed that under conditions where the substrate concentration is high, relative to that of the enzyme, most of the enzyme is complexed, so that the rate of reaction is independent of the substrate concentration and proportional to the complex concentration. At sufficiently low substrate concentration, the rate of reaction is proportional to the concentration of the substrate.

Enzymes consist of various sequences of amino acids linked together in polypeptide chains. Various functional groups along the chain such as COOH, OH and NH_2 give the enzymes a strong polar and hydrophilic nature. This limits their ability to complex with certain types of polymers, especially those which are insoluble in water or which are water-repellent.

5.2.4 *In vitro* and *in vivo* degradation of polymeric materials is strongly dependent on factors related to the basic polymer chemical and physical characteristics and on the processing and/or treatment history.

Factors related to the basic characteristics of the polymer include relative molecular mass, polydispersity, chemical structure, polarity, molecular conformation and crystallinity.

Factors related to processing/treatment of the polymer include molecular orientation and crystallinity, presence of impurities/additives, presence of voids/defects, blending, geometric factors, process/treatment-induced decrease in relative molecular mass, environment (e.g. pH level), site of implantation, load, temperature and humidity.

5.2.5 Polymeric medical products and devices are produced from polymers and plastics using standard polymer processing techniques, mainly injection-moulding, melt-extrusion, compression-moulding, vacuum-forming, blow-moulding, etc.

These processes are performed at elevated temperatures, and often at high shear rates, which may lead to at least a partial material degradation. Processing may also cause accumulation of stresses in a device which in a biological environment may lead to environmental stress-cracking. In some cases, devices are treated using various liquids (e.g. for material extraction or solvent-welding) that also induce changes in the material/device. Additives in the material or device such as plasticizers, lubricants, colorants, light stabilizers and antioxidants may enhance or inhibit degradation, mainly due to leaching.

5.2.6 Medical materials and devices are usually supplied in a sterile form. Sterilization is performed using physical or chemical methods, e.g. heat, steam, gas (ethylene oxide) or ionizing radiation, mainly gamma or beta. Each of these sterilization processes may have an effect on the degradation of the material. Radiation sterilization using high energy radiation requires doses in the range of 1,5 Mrad to 3,5 Mrad with the most common dose rate being 2,5 Mrad. This, in some polymers, could result in various extents of crosslinking and/or degradation of the polymer or changes in the additives used in the polymer processing.

5.2.7 Exposure of a material or device to UV light can also result in a partial degradation.

Steam sterilization may cause degradation of devices made from polymers, mainly due to hydrolysis. Ethylene oxide sterilization may lead to formation of byproducts in a sterilized material, while the gas residues in poorly degassed objects may interact with the physiological media in contact with the device. Dry heat sterilization at temperatures up to 180 °C may lead to thermal-oxidative degradation.

5.2.8 In general, the rate of degradation of synthetic polymers decreases with an increase in relative molecular mass and decrease in polydispersity or an increase in crystallinity and chain orientation.

Copolymers usually degrade faster than homopolymers from the same family; linear polymers are more susceptible to degradation than those which are crosslinked. The rate of degradation of polymers is

greater for blends, porous materials, materials with voids, impurities, additives, etc., than for pure solid materials.

Usually, large, bulky implants degrade slower than implants with small cross-sections. Loading or stressing the material enhances its degradation. An acidic or alkaline environment accelerates hydrolysis and/or oxidative degradation of some polymers, e.g. polyamides or polyesters. Degradation is enhanced at elevated temperatures.

5.3 Ceramics

5.3.1 Chemical dissolution of ceramics

In vitro degradation of ceramics is related to the specific chemistry involved. Mechanisms of *in vitro* degradation for glass, calcium phosphates, alumina/zirconia and cements are discussed in 5.3.1.1 to 5.3.1.4.

5.3.1.1 Glass

In the case of bioactive glasses and glass ceramics, the corrosion reactions in general can be described by two main reactions: ion exchange and network dissolution. In the former reaction alkali ions in the glass are replaced by H^+ or H_3O^+ from the solution. In the second reaction, hydrolysis occurs resulting in network dissolution through the attack of hydroxyl ions on the mixed silica-alumina-phosphate-network. Some bioactive glasses were corrosion-tested in deionized water, tris-buffer, tris-buffer made isotonic with NaCl, tris-buffer containing citrate and simulated extracellular fluid. These corrosion tests were carried out in polystyrene containers at 37 °C ± 0,5 °C and a pH of 7,2. When the solubility product within the closed vessel was exceeded, calcium phosphate crystals of an apatitic nature were precipitated on the surface of the bioactive glasses. The presence of citric acid enhanced alkali extraction by complexation of cations and inhibited calcium phosphate reprecipitation.

5.3.1.2 Calcium phosphates

When calcium phosphate ceramics with a Ca/P molar ratio greater than one are brought into contact with aqueous solutions between pH 4 and pH 9 in a closed system, they are transformed sooner or later into apatite-like materials, except for beta tri-calcium phosphate (β-TCP) and magnesium containing β-TCP. These retain their structure and become covered with a layer of whitlockite and magnesium whitlockite respectively. Hydroxyapatite or fluorapatite also both retain their normal apatite structure. All calcium phosphates thus dissolve up to the point where the surrounding fluid becomes saturated with the relevant apatite or the relevant form of β-TCP. Body fluids are supersaturated with apatite and are, in fact, close to equilibrium with octacalcium phosphate.

5.3.1.3 Alumina and zirconia

Aluminium and zirconium ions form fairly strong complexes in aqueous solutions with hydroxyl, fluoride and phosphate ions, so that it must be expected that even dense alumina or zirconia dissolves somewhat in extracellular fluids.

5.3.1.4 Inorganic cements

Recently, cements have been developed by reaction of an acidic calcium phosphate such as brushite or octa-calcium phosphate (OCP) with a basic calcium phosphate like tetracalcium phosphate. During their setting, an apatite-like material is formed. As these cements consist of calcium phosphates, it is to be expected that in contact with aqueous solutions they behave like other calcium phosphates. First dissolution occurs which may be followed by reprecipitation of another calcium phosphate.

Other cements such as silicate, zinc polyacrylate and zinc oxide-eugenol cements and glass ionomers undergo measurable ionic exchange in aqueous solutions.

5.3.2 Mechanical degradation of ceramic materials

Local stress at the implant/tissue interface can be caused by several factors, such as a difference in elastic modulus between implant and surrounding tissue or body fluids, loading of a prosthesis, interparticle movement, and the presence of bordering muscle tissue. There is considerable controversy about the interface behaviour of biomaterials, emphasizing the importance of defining the primary load at the biomaterial/tissue interface. Ceramics are as susceptible to local stress effects as any other biomaterial. This can be seen for ceramics such as aluminium oxide, glass ceramic and calcium phosphates, where intramuscular implantation involves divergence of the elastic modulus with local stress caused by bordering muscle tissue. The wear caused by local stress is of particular concern for the biocompatibility of a ceramic since it strongly affects the inflammatory response. Aluminium oxide is considered to be a highly inert implant material, not susceptible to degradation and/or wear. Nevertheless, ceramic particles have been seen in phagocytes bordering solid bodies of aluminium oxide when im-

planted intra-muscularly. Ceramic debris is created by wear, and aluminium-rich inclusions in tissues surrounding aluminium oxide in bone have been found. Wear debris has been shown to be phagocytosed by macrophages in dogs and in humans. The effects caused by wear seem generally limited, but several studies have demonstrated that much more than solid aluminium oxide particles give rise to an inflammatory response. Calcium phosphates, such as hydroxyapatite and tricalcium phosphate, have little flexural strength and were initially not designed for load-bearing applications, which would cause local stress and associated wear. However, in recent years these ceramics are increasingly applied as coatings on load-bearing orthopaedic or dental devices which emphasizes the importance of studying the effect of calcium phosphate particles on the surrounding tissues.

5.3.3 Cellular and enzymatic degradation of ceramic materials

The role of cells and enzymes in ceramic material degradation is at present unclear. Aluminium oxides have been considered to be highly inert implant materials, resistant to degradation and/or wear. However, ceramic particles have been observed in phagocytes by histopathological methods. Microprobe analysis has demonstrated the presence of aluminium in tissue surrounding aluminium oxide implants. Aluminium oxide particles may cause a release of lysosomal enzymes and the cytoplasmic enzyme LDH. Studies performed to date generally show that calcium phosphate particles do not cause significant inflammatory response.

However, the presence of unwanted impurities in a calcium phosphate material may significantly affect biological response testing of these materials.

Clinical reports have suggested increased levels of degradation of calcium phosphates and glass ceramics associated with infection. This may be related to the interaction between the microorganisms and the ceramics or to the tissue inflammation associated with infection.

The release of trace elements may be promoted by enzymatic and other cellular reactions. Five different types of calcium phosphate ceramics have been investigated in several experimental conditions and each gave rise to the accumulation of trace elements in addition to larger amounts of calcium and phosphorus in surrounding phagocytes. Most of these trace elements were derived from the implant. Apparently impurities enclosed in ceramics are phagocytized in macrophages and multinucleated cells, and accumulated. This means that unwanted impurities in ceramics need to be avoided, especially for resorbable ceramics.

5.4 Carbons

Degradation of carbon structures can be due either to mechanical or chemical action, or a combination of the two. With few exceptions, carbon and graphite show little reaction with water or other low-temperature liquid solutions. The primary limitations of carbon and graphite result from their brittle nature. The primary mechanical mechanisms of carbon degradation are wear, fatigue, overload fractures, fatigue/fracture, spalling of coating and cavitation erosion. The principal mechanism of chemical degradation of carbon is oxidation.

5.5 Composites

The degradation of composites takes place according to the degradation mechanisms of the constituents used in the device (see table 4). These mechanisms have been discussed in more detail in the clauses of this Technical Report which deal with the component parts of the composite, i.e. polymers, ceramics and metals. It should be pointed out that the degradation of the constituent components in the composite can be modulated by the composite specific mechanisms which are interface-related, due to the interaction of different materials.

The processing methods used for fibre preparation may affect the degradation of composites as they influence crystallinity, molecular mass, etc. of the resulting fibres. Abrasion plays an important role in the performance of composites used in medical devices.

5.5.1 Chemical and biological degradation of composites

The following mechanisms can be involved in the degradation of composites:

a) adsorption of liquids:

   — to the surface of the device,

   — at the reinforcing material/matrix interface;

b) absorption and swelling of the matrix and/or the reinforcement;

c) migration along the reinforcing material/matrix interface;

d) chemical reaction between the liquids and at least one component of the composite;

e) reaction of the composite surface with the biological environment;

f) secondary reactions of one component of the device with the degradation products of another component;

g) enhancement of degradation by environmental stresses.

In reinforced materials, local stresses may be generated, and sometimes are desired. They are generated, for example, through the manufacturing process, or by the use of materials with different coefficients of thermal expansion at the reinforcement/matrix interface. Swelling of matrix and/or the reinforcement material may lead to local stresses at the interface. These local stresses lead to enhanced degradation.

The reinforcing material, especially when there is insufficient bonding with the matrix, can be considered as an "impurity" or "defect" in the matrix which initiates degradation.

5.5.2 Mechanical degradation process

Two types of abrasion are considered:

a) surface abrasion:

- friction by macromotion during use (dental implants, hip joints),
- friction at implant/tissue interface by micromotion;

b) interface abrasion by motion between matrix and reinforcing material.

Surface abrasion of the composite may expose reinforcing material and result in changes of the chemical, biological and mechanical performance of the device.

A special problem with composites is the abrasion at the interface of the matrix and the reinforcing material. It results from the use of components with different moduli within the composite which allows relative movement under stress. This may be avoided by improving the bond of the matrix to the reinforcing material.

5.6 Metals and alloys

5.6.1 The principal mode of degradation of metals and alloys in the physiological environment is corrosion. Corrosion susceptibility of metals and alloys is largely determined by their composition and structure. Table 5 classifies medically used metals and alloys into three groups based on their electrochemical status and ability to form oxide layers.

- The first group of alloys shown in table 5 is based on chemically stable, noble metals such as gold, platinum and palladium.
- The second group of alloys is based on metals which tend to form chemically stable oxide layers such as titanium, niobium or tantalum.
- The third group of alloys, whose major constituent does not produce a protective surface layer, is protected by a minor alloy constituent that does form a chemically stable oxide.

Table 4 — Influences on degradation rate of composites

Material	Processing/treatment	Implantation
Chemical Relative molecular mass Polydispersity Chemical structure Chain regularity **Physical** Molecular orientation Crystallinity Hydrophilicity Swelling behaviour	Formulation Impurities Additives Voids defects Temperatures Thermal cycles Pressure Humidity Sterilization Environmental stresses Interface bonds Cleaning Annealing Finishing	Implantation site pH Loading Micromotion Infection and inflammation

Table 5 — Corrosion susceptibility of medically used metals and alloys

	Major alloy constituent	Reference	Composition and form
1	Noble metal alloys		
	Gold alloys		
	High gold alloys	ISO 1562 ISO 9693	> 75 % (*m/m*) Au + Pt-metals/cast
	Low gold alloys	ISO 8891 ISO 9693	> 25 % (*m/m*) < 75 % (*m/m*) Au + Pt-metals/cast
	Palladium alloys		
	Palladium-silver alloys	ISO 8891 ISO 9693	> 25 % (*m/m*) < 75 % (*m/m*) Au + Pt-metals/cast
	Palladium-copper alloys	ISO 8891 ISO 9693	> 25 % (*m/m*) < 75 % (*m/m*) Au + Pt-metals/cast
	Platinum alloys		wrought
	Silver alloys		
	Silver-copper alloys	ISO 8891	> 25 % (*m/m*) < 75 % (*m/m*) Au + Pt-metals/cast
	Silver-tin alloys	ISO 1559	alloys for amalgams
2	Metals and alloys which form protective oxide layer		
	Titanium		
	Titanium C.P.	ISO 5832-2	cast
	Titanium alloys	ISO 5832-3 ISO 150-1 N	Ti6aL4V, wrought TiAlNb
	Niobium		
	Niobium C.P.		
	Niobium alloys		
	Tantalum		
	Tantalum C.P.		
3	Alloys with oxide layer of constituent		
	Ferrous alloys		
	Stainless steel		

5.6.2 Exposure of metals/alloys to fluids often leads to the oxidation of specific metal atoms. As a result metal ions are released from the surface of the material into the surrounding tissues and fluids. This may cause local and systemic responses.

5.6.3 The chemical and electrochemical corrosion of a material made from several components, such as a metallic alloy, within a complex electrolytic medium, such as saliva or plasma, is generally due to several oxidation processes occurring at the same time.

5.6.4 The synergistic interaction between the chemical and electrochemical environments and mechanical stress can lead to increased amounts of metal ions being released into the biological system. These interactions fall into four basic categories: stress corrosion, fatigue corrosion, fretting corrosion and crevice corrosion.

5.6.4.1 Stress corrosion causes the formation of a crack in the presence of stress. It can be a self-accelerating phenomenon that may lead to total material fracture. Stress corrosion has been related to a variety of mechanisms depending on the alloy and environment involved. Some materials have exhibited stress corrosion cracking in environments similar to the chemical environment of the human body.

5.6.4.2 Fatigue corrosion leads to a decrease in the fatigue strength of a medical device. The mechanism of fatigue corrosion is similar to that of stress corrosion cracking, but with a more active role for the repetitive loading. It can also be enhanced by subsequent corrosion due to stress concentration arising from a corrosion pit.

5.6.4.3 Fretting corrosion results from the combined effects of surface wear by abrasion and the corrosive attack by the physiological environment. The abrasive wear leads to the rupture of the corrosion-protecting passivating oxide layer, exposing the active alloy surface to corrosion attack.

5.6.4.4 Crevice corrosion results in an accelerated corrosion rate within a fissure, crack or gap.

5.7 Coatings

5.7.1 The mechanisms of coating degradation have been discussed under the clauses dealing with each major material group (i.e. polymers, ceramics, composites and metals).

For a coating material, the nature of the interface between the coating and the substrate will significantly affect its stability. With laminated coatings, the interfacial strength between the layers is also considered important.

5.7.2 The degradation of coatings results in chemical and physical changes which affect their properties and performance. Such changes may lead to crack initiation and propagation, fracture, debonding, chemical transformations and in some cases, dissolution.

The degradation of coating materials is influenced by the following factors:

a) mechanical:
   - — static stresses: compression, tension, torsion,
   - — dynamic stresses: cyclic fatigue;

b) chemical or physical:
   - — hydrolysis,
   - — oxidation-reduction,
   - — dissolution,
   - — photo or high-energy radiation,
   - — temperature,
   - — electrical current;

c) biological:
   - — cellular activity,
   - — inflammation,
   - — infection;

d) others:
   - — processing,
   - — defects (pinholes),
   - — ageing.

6 Techniques for *in vitro* degradation testing of medical materials and devices

Techniques for degradation tests of medical materials and devices are dependent upon the unique chemical, physical and mechanical properties of the material or device under investigation.

In general, *in vitro* degradation tests should be carried out under conditions which closely simulate the en-

vironment in which the material and/or device is going to be used. The selection of tests should reflect the intended function of the material/device.

All the tests shall be carried out on the final, ready-for-use materials or devices selected in accordance with accepted scientific principles. The requirements for sample preparation and reference materials in ISO 10993-3, ISO 10993-5, ISO 10993-6, ISO 10993-7 and ISO 10993-12 shall be followed.

It is proposed that research working groups and the industries dealing with the same device standardize their specific simulation tests in order to get a comparability of their results.

If a test medium is to be used for elution testing, a minimum of 110 ml of buffer solution should be sampled.

6.1 Polymers

6.1.1 General

There are no standards available which describe methods for testing *in vitro* degradation of polymeric medical materials and/or devices. Various test procedures are used by different groups depending on the class of material dealt with and the application. Current *in vitro* degradation testing for polymers is generally conducted at 37 °C ± 1 °C in different buffers at various pH-values. The ageing medium can also contain enzymes and ions of elements which are expected to be present in the *in vivo* environment. It should be noted that *in vitro* degradation tests carried out at temperatures above the glass transition which itself is above ambient or body temperature may affect the crystallinity and rate of degradation of the material.

6.1.2 Accelerated time test

The following is a proposed accelerated test for assessing degradation of polymeric materials.

Samples used for testing should be dried to constant mass before testing at conditions suitable for the particular material or device. Tests should be carried out at pH-value of 7,4 in the phosphate buffer, at temperatures of 50 °C ± 1 °C or 80 °C ± 1 °C. Temperatures selected for testing will depend on the material or device. Materials or devices should be tested for 1, 3, 6, 15, 30 and 60 days.

WARNING — The highest temperature used must never exceed the melt flow temperature of the polymer being tested.

Ensure that the drying process does not affect the material to any degree, e.g. change of crystallinity.

Phosphate buffer contains: 0,5 mol (68,08 g/l) of KH_2PO_4 and 0,5 mol (89,07 g/l) of Na_2HPO_4 in 0,9 % of saline (NaCl) solution in bidistilled water. Salts used for preparation of the buffer should be of analytical grade and should be dried to constant mass.

6.1.3 Real-time test

Samples should be dried to constant mass before testing. The temperature selected for drying samples should not exceed the temperature at which the material or device may undergo irreversible changes, e.g. melting, flowing or degradation. Degradation tests should be carried out at 37 °C ± 1 °C in the phosphate buffer solution cited in 6.1.2.

Materials and devices should be tested *in vitro* for 1, 2, 4, 12 and 26 weeks. It is possible that microbiological growth could cause degradation to occur in the test materials. The decision about the duration of the *in vitro* degradation test should take into account the time the material or device will be in contact with the tissues and body fluids.

The amount of material used for *in vitro* degradation tests should be large enough to allow further characterization. Usually, individual samples should be as large as 1 g to 5 g, depending on the number of characterization tests to be conducted.

The ratio between the surface area of the device and the volume of buffer solution shall be in the range of 1 cm^2/ml to 6 cm^2/ml.

6.2 Ceramics

6.2.1 Standard methods

At present there exist no standard methods for testing the degradation of bioceramics other than dental porcelain. Inorganic cements applied as dental cements are subject to tests on "solubility and disintegration", some recommending the use of distilled water as the medium for degradation, and one a mixture of 4 % (*V/V*) of acetic acid in distilled or deionized water.

6.2.2 *In vitro* degradation testing

Degradation tests for bioceramics and inorganic biocements should be done in an environment which simulates the *in vivo* conditions as far as physicochemical parameters are concerned. The average pH of extracellular fluid is 7,4. However, near glycolytically active cell, the extracellular fluid is satu-

rated with carbon dioxide and the pH may be as low as 6. Moreover, osteoclasts and macrophages excrete lactic acid in amounts which could lower the local pH to 4.

In order to simulate these environments, exposure of the test samples to three different media is proposed. The first exposure medium (solution A) is an aqueous solution which resembles human extracellular fluid without calcium, magnesium or phosphates:

100 mM NaCl

45 mM $NaHCO_3$

2 mM K_2CO_3

The pH of this medium at 37 °C ± 1 °C is approximately 7,4.

The second aqueous exposure medium is identical in composition to the first, but in combination with continuous bubbling of carbon dioxide (solution B).

To prepare a third exposure medium (solution C), the pH of solution B is lowered to 4 by adding lactic acid and maintained during the test.

The proposed degradation tests for bioceramics may be carried out as follows. Nine samples of the bioceramic or the inorganic biocement having the form of a cylinder with a diameter of 6 mm and a height of 12 mm are prepared and weighed. One hour after preparation, three samples are exposed to solution A. Each sample is suspended from a cotton string in a polyethylene beaker containing 100 ml of solution so that the sample is positioned in the middle of the solution. Similarly, three remaining samples are exposed to solution B and the other three samples to solution C. The exposure time is 72 h and the temperature is maintained at 37 °C ± 1 °C. After exposure, evaluate the degradation products as follows.

Centrifuge the solution to remove any debris generated due to the degradation test and weigh the debris. Homogenize and analyse the remaining solution for the oxides and fluorides originally contained in the bioceramic or cement. Measurements of sodium or potassium oxides should not be done as these results would not be meaningful. The exposed samples should be dried and weighed to determine loss of mass.

6.3 Composites

6.3.1 General

No standards are available describing methods for testing *in vitro* degradation of composite medical materials and/or devices. Specific test procedures which have been used depend on the class of material and address the application.

Because of the multiphase character of the composites, the study of the interface is essential. The diffusion of liquids into the device and reinforcing material may require extended exposure times. To accelerate this procedure, it is necessary to increase exposure of the reinforcing material to the aqueous media, e.g. by cutting the device into parts.

6.3.2 Accelerated test

The tests described should reference the tests addressed in the material class appropriate to the composite constituents.

6.3.3 Real-time test

These tests should reference the tests addressed in the material class appropriate to the composite constituents.

6.4 Metals and alloys

6.4.1 *In vitro* test procedures used should simulate to as large an extent as possible the natural physiological conditions. It is recognized that *in vitro* testing is necessarily based on relatively short-term experiments from which extrapolations have been made to predict the long-term performance of materials or devices. In these tests, the physiological environment has to be simulated by artificial solutions (see annex C). Many investigations have been reported on the composition of specific body fluids and the influence of the different constituents on the degradation processes of metals and alloys. Complicating this situation is the fact that some body fluids vary in composition and pH depending on various physiological factors. Electrochemical test procedures are frequently used to provide data on the biostability of metallic biomaterials.

NOTE 3 Example standard methods include NF S 90-701, NF S 91-141, ASTM F-746 and ASTM F-897.

6.4.2 These test methods estimate the electrochemical corrosion-resistance of a metallic biomaterial in a biological environment. Following immersion tests, analytical chemistry procedures are employed

to quantify the dissolved metal ions in the test solutions, which gives a direct indication of the corrosion susceptibility of the material.

The standards cited in 6.4.1 suggest the following three *in vitro* electrochemical methods for corrosion susceptibility assessment:

— the potentiostatic polarization method, equal to chronoamperometry with a constant potential;

— the potentiodynamic polarization method, equal to voltamperometry with a linear variation of the potential with or without cycling; and

— the analysis of the electrolyte and the corroded material surface after the test at a definite potential for a definite time at a definite temperature.

6.5 Coatings

Degradation of coatings should be evaluated at the three levels of coating, substrate and interface.

The exposure solutions and extraction conditions for coating materials is governed by the individual coating component material compositions and the tests described for those materials.

The choice of tests to evaluate a given coating depends on the use of the implant itself and cannot be considered in this Technical Report.

7 Methods for evaluation of degradation products from *in vitro* studies

The assessment of the biological response to a medical material or device depends in part on a knowledge of the degradation products. Therefore, the characterization of medical materials/devices before and after exposure to selected test solutions provides important information and data on the degradation mechanisms and products. Analysis of the extracts of these materials and devices yields both qualitative and quantitative information on the degradation products.

7.1 Chemical analyses of extracts

7.1.1 Polymers

After extraction, test samples should be carefully removed, and the test solution subjected to the appropriate chemical analysis. The selection of the chemical analysis should be based on known leachable fractions and all possible modes of degradation for the specific polymer being evaluated. These may include additives such as plasticizers and antioxidants, monomers or other low relative molecular mass moieties, polymer fragments, etc.

7.1.2 Ceramics

No standards currently exist for chemical analyses of degradation products from ceramics exposed in biological environments.

7.1.3 Composites

For composites whose major constituent is a polymer, the general procedure described in 7.1.1 applies. The techniques for handling ceramic composites is noted in 7.1.2 and for metallic composites in 7.1.4.

7.1.4 Metals and alloys

Both the corrosion immersion test and the potentiostatic polarization method with the analytical determination of dissolved metal ions give first indications of the degradation of a metal or an alloy surface. The amount and type of metal ions released into this electrolyte depend on the following factors: pH, temperature, time, hydrodynamic conditions, oxygen levels in the electrolyte, etc.

Following the immersion tests described in 6.4, analytical procedures are employed to determine the quantity of dissolved metal ions in the test solution.

NOTE 4 Methods used to make these determinations can be found in standard analytical chemistry texts and are also provided in standards such as ASTM E-60 (03.05), *Photometric and spectrophotometric methods*, ASTM A751, *Steel products methods practices and definitions*, ASTM C-1111 (12.01), *Trace, minor, major elements in waste streams by emission spectroscopy*.

7.1.5 Coatings

As noted earlier, metal device coatings may be in the form of monolayer or multilayer structures. The extraction of coatings to recover degradation products may present special analytical problems which require specialized extraction procedures. For the purposes of selecting appropriate extraction techniques, coatings need to be classified according to their chemical composition. Then the extraction procedure will be governed by the coating material characteristics: polymeric, metallic, ceramic, composite.

7.2 Material/device analysis

7.2.1 Polymers

7.2.1.1 Relative molecular mass changes

Changes of relative molecular mass of the polymeric material/device due to *in vitro* degradation tests or extraction can be assessed by viscosity measurements (7.2.1.2), gel permeation chromatography (GPC) (7.2.1.3) or other techniques.

7.2.1.2 Viscosity measurements

Relative viscosity of a polymer should be measured in the Ubbelohde or Canon-Fenske type viscometer at an appropriate temperature to within ± 0,02 °C, using solvents adequate for a particular polymer. As relative viscosity is sufficient to characterize the relative molecular mass of a material/device, it is not necessary to calculate the absolute values of viscosity-average molecular mass.

Viscometers used for measurements should be selected in such a way that the flow time of the solvent in the viscometer is in the range of 90 s to 120 s.

Concentrations of polymers in solution should be in the range of 0,05 g/100 ml to 0,5 g/100 ml. (See ASTM D-20.)

7.2.1.3 Gel permeation chromatography (GPC, SEC)

The number-average molecular mass, mass-average molecular mass and polydispersity (molecular mass distribution) of a material/device can be estimated using gel permeation chromatography. The polymer should be dissolved in a suitable solvent, if possible the same as is used for viscosity measurements. Temperatures at which measurements are carried out will depend on the polymer used.

7.2.1.4 Mass changes

During *in vitro* degradation tests and/or extraction, the mass of the material/device may undergo changes. These changes may be monitored by comparing the masses of the samples dried to constant mass before and after the test. The mass loss or gain is given as a difference between the sample mass before and after the *in vitro* degradation test.

7.2.1.5 Thermal properties

Properties of a material/device (melting temperature, heat of melting, glass transition temperature), should be characterized using a standard differential scanning calorimeter (DSC), at a scanning rate in the range of 5 °C/min to 20 °C/min, and preferably 10 °C/min. Sample mass should be in the range of 5 mg to 20 mg, depending on the heat effect expected (crystalline against amorphous polymers).

7.2.1.6 Changes in chemical structure

The chemical structure of the material/device before and after degradation/extraction tests, characteristics of the byproducts formed upon degradation, and the degradation products formed in the extract can be assessed using suitable techniques such as infrared spectroscopy (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), gas chromatography (GC), high pressure liquid chromatography (HPLC), ESCA, SIMS. Standard sample preparation techniques should be used with the evaluation methods mentioned above.

7.2.1.7 Changes on surface and in bulk (volume) of material/device

Changes on the material surface such as seen with environmental stress-cracking, surface erosion due to leaching, etc., and changes within the bulk (volume) of the material such as cracks, voids, defects created upon leaching of low relative molecular mass components, can be assessed using scanning electron microscopy (SEM), ESCA, SIMS, etc. Changes within the material volume should be observed on the fracture surface, produced by breaking the material frozen in liquid nitrogen.

7.2.1.8 Impurities

For the study of impurities of low concentrations and of composition patterns, the ion-microprobe is used. Further techniques of surface analysis such as photoelectron spectroscopy and Auger electron spectroscopy are used for the material.

Certain X-ray diffraction techniques, which analyse the crystallographic structure in the surface layer, provide measures of stress and of work-hardening.

7.2.2 Ceramics

7.2.2.1 Characterization of the samples before and after exposure to the three solutions identified in 6.2 may be as follows:

a) mass loss by the gravimetric method;

b) compression strength;

c) diametral tensile strength.

7.2.2.2 Surface, reprecipitated products and for example particulate debris should be studied by:

a) X-ray diffraction;

b) infrared absorption or reflection spectroscopy;

c) optical microscopy;

d) SEM;

e) electron microprobe analysis;

f) SIMS, ESCA.

7.2.3 Composites

7.2.3.1 To measure degradation of composites, following exposure to immersion solutions, the exposed material has to be tested in comparison to unexposed material for changes in relative molecular mass and mass.

The chemical analyses should be performed on each component of the composite.

7.2.3.2 Tests for the following properties may be found in 7.2.1:

— relative molecular mass changes (viscometry/GPC);

— mass changes;

— thermal properties;

— changes in chemical structure;

— surface and bulk (volume) evaluation (SEM).

7.2.4 Metals and alloys

7.2.4.1 Surface characterization before corrosion testing

Before each electrochemical test the samples should be examined under an optical microscope to visualize heterogeneities (pores, pits, grain boundaries, precipitates, etc.) Scanning Electron Microscopy (SEM), will complement optical microscopy observations (roughness, cracks, polishing scratches, etc.).

On polished surfaces, SEM will allow the visualization of the impurities and precipitates which can be quantitatively analysed in the range of micrometres. This is done with X-ray diffractometry, scanning electron microscopy and electron microprobe analysis. Special problems may call for more complex analysis techniques such as SIMS, ESCA, etc.

7.2.4.2 Sample characterization after corrosion testing

An investigation under an optical or scanning electron microscope allows the evaluation of the corrosion effects: presence of pits, intergranular and intragranular corrosion, formation of passivation layers and of corrosion products constituting depositions more or less sticking to the metal surface.

Identifying the corrosion products (oxides, chlorides, hydroxides, etc.) is complex. However, a precise knowledge of these products is needed for further biological evaluation.

7.2.5 Coatings

Techniques for evaluating the degradation of coatings are dependent on the nature of the coating medium used (polymer, carbon, metal). Methods for evaluating degradation of coatings should refer to the appropriate material class section clauses.

8 Identification and quantification of *in vivo* degradation products from medical implants

The majority of implants are intended to remain in the body for prolonged periods of time and perform their intended functions without degradation. Thus, the long-term stability of materials and implants in the physiological environment is of critical importance to ensure safety and effectiveness. However, "biostability" is a relative term because (perhaps with the exception of pyrolytic carbons), there are no fully stable materials. This being the case, the following problems arise:

a) how much degradation takes place during a given period of time;

b) what are the degradation products;

c) what is the origin of the degradation products; (e.g., impurities, additives, corrosion products, bulk polymer);

d) qualitative and quantitative assessment of degradation products and leachables in adjacent tissues and in distant organs.

The problem is further complicated by the presence of metabolic products originating from the degradation.

8.1 Factors affecting *in vivo* degradation of implant materials/devices

Mechanisms of degradation of the different classes of materials have been discussed in previous clauses and will not be covered here.

Biological factors which could contribute to material/device degradation are briefly discussed. All implanted materials cause inflammatory reactions to various extents. Inflammatory reactions precede degradation processes and include:

— activation of proteins (e.g., complement, plasma components);

— denaturation of surrounding tissue proteins;

— migration of neutrophils and monocytes into the implant/tissue interface by chemotaxis or chemokinesis;

— differentiation of monocytes into macrophages;

— phagocytosis of cell debris and small particles (< 60 µm, measured along the longest axis) of foreign materials by macrophages and foreign body giant cells;

— generation of superoxide anion intermediates and hydrogen peroxide by some activated and phagocytosing neutrophils and macrophages leading to possible surface erosion (degradation) of polymeric materials.

8.2 Degradation products in adjacent tissues

Although many biomedical materials and implants should perform safely and reliably for prolonged periods of time while in the body, there are no totally "stable" materials (with the possible exception of carbons) when in contact with the corrosive physiological environment. Thus, some degradation of implanted materials is the inevitable consequence of prolonged exposure in the body. Material/device degradation *in vivo*, which involves all types of processes in the living organism, leads to changes in their physical, electrical, chemical and biological properties.

The identification of degradation products from implants is scantily covered in the literature. For example, ASTM F501-87, *Standard Practice of Analysis of Retrieved Metallic Orthopedic Implants*, under clause 6.1.2-2, Histopathological Observation, notes only the use of polarized light for the detection of degradation particles.

8.3 Comments on specific test procedures

8.3.1 Light microscopy and associated techniques

Some identifications can be made on single particles of 10 µm or less measured along the largest axis. In addition to the usual transmission bright field microscopy, several other techniques are also used, including transmitted and reflected polarized light microscopy for characterization of birefringent particles; transmitted and reflected phase contrast microscopy, applicable where the refractive indices of various component materials are similar; and transmitted and reflected differential interference contrast (Nomarski) microscopy for surface characterizations. Practical resolution is of the order of 0,2 µm.

8.3.2 Transmission electron microscopy

This technique is used for the characterization of materials based on their morphology and electron diffraction with magnifications in the range of × 50 to × 500 000, with practical resolution in the range of 0,14 nm to 0,5 nm.

8.3.3 Scanning electron microscopy

A particular imaging mode of transmission electron microscopy is used in scanning transmission electron microscopy and scanning electron microscopy. The latter offers a magnification range of × 15 to × 400 000, with practical limit of resolution of about 2 nm. It is particularly useful in surface analysis in conjunction with Energy Dispersive X-ray Analysis (EDAX) of micrometre-sized particles.

8.3.4 Electron probe analysis

This technique is used for the elemental analysis and characterization of materials and particles as small as about 1 µm, and is applicable to elements with atomic numbers greater than or equal to 5.

8.3.5 Neutron activation analysis

This technique which can be used for 30 (or more) elements has a quantitative limit of µ/kg concentrations.

8.3.6 Atomic absorption spectroscopy

This technique is used for the determination of metallic elements in inorganic and organic materials. The detection limit is 0,1 ppm to 20 ppm for more than 30 elements.

8.3.7 Inductively coupled plasma-mass spectrometry

This technique is a sophisticated method to determine elements in solid and liquid samples.

8.3.8 Microincineration

This technique permits the detection of metals in tissues arising from metallic implants. Briefly stated, it involves the microincineration of tissue slices in a specially constructed furnace and their subsequent exposure to sulfuric acid vapour to convert any metal present in the tissue to the sulfate. Tissue slides are then stained for the particular metal (e.g., tannic acid for titanium (yellow colour); rubeanic acid for cobalt (orange colour).

8.3.9 Thin layer chromatography (TLC)

This technique is used primarily in the separation and quantitative determination of components in mixtures of components. The detection limits for trace components are generally in the range of 0,1 ng/spot to 100 ng/spot for UV or visible absorption and 0,01 ng/spot to 10 ng/spot for fluorescence spectrophotometry.

8.3.10 Gas-liquid chromatography (GLC)

This technique is used in the qualitative and quantitative analysis of mixtures of organic and inorganic components. Sample sizes ranging from 10^{-2} g to 10^{-14} g can be used. Major components may be analysed with accuracy of 98 % to 100 % and with precision of nearly $\pm$ 0,1 %.

8.3.11 Additional applicable techniques

Additional techniques which may be relevant are those such as the following:

SIMS Secondary ion mass spectrometry

ESCA Electron spectroscopy for chemical analysis

Auger Auger spectroscopy

FTIR Fourier transform infrared spectroscopy

NMR Nuclear magnetic resonance

GPC Gel permeation chromatography (size-exclusion chromatography)

TGA Thermal gravimetric analysis

HPLC High performance liquid chromatography

8.4 Proposed test protocol: degradation products of implanted materials in adjacent tissues

The following flow chart presentations indicate the proposed protocols.

a) Polymers

- preparation and sectioning
- specific staining
- optical microscopy:
  - → bright field
  - → polarized light
  - → reflected phase contrast
  - → reflected differential interference contrast (Nomarski)
- scanning electron microscopy
- transmission electron microscopy
- thin layer chromatography
- gas-liquid chromatography
- spectroscopic techniques
- atomic absorption
- microincineration

b) Composites (fibres embedded in polymers)

- preparation and sectioning
- specific staining
- optical microscopy:
  - → bright field
  - → polarized light
  - → reflected phase contrast
  - → reflected differential interference contrast (Nomarski)
- melting points of polymeric debris: hot stage microscope and differential scanning calorimetry (DSC)
- scanning electron microscopy
- transmission electron microscopy
- thin layer chromatography
- gas-liquid chromatography
- spectroscopic techniques
- atomic absorption
- microincineration

c) Metals and alloys

- sterilized tissue slice preparation
- optical microscopy: bright field
- optical microscopy: reflected phase contrast
  - stainless steel/titanium/cobalt-chromium alloys
    - hematoxylin-eosin stain
      - Perls' stain
- neutron activation analysis
- electron probe microanalysis
- atomic absorption spectroscopy
- energy dispersive X-ray microanalysis - SEM [or inductively coupled plasma - mass spectrometer]
- microincineration

d) Ceramics

- preparation and sectioning
- staining: hematoxylin-eosin and van Gieson
- optical microscopy: bright field
- optical microscopy: polarized light
- scanning electron microscopy
- electron probe microscopy
- neutron activation analysis

e) Coated or multi-combined materials

- preparation and sectioning
- optical microscopy: bright field
- optical microscopy: polarized light
  - → polymer coating on polymer:
    - follow a) - Polymers
  - → polymer coating on metals/alloys:
    - [• follow c) - Metals/alloys]
  - → Ceramic (including carbon) coating on metals (or graphite)
    - follow d) - Ceramics
    - [• follow c) - Metals]

[ ] = alternative, as needed

Annex A
(informative)

Analytical characterization of resorbable polyesters based on lactic and glycolic acid

A.1 Introduction

A.1.1 Analytical methods, which should be used for *in vitro* evaluation of degradation products, will depend on the material under investigation. Thus, no general analytical scheme, which could be used universally for all the materials/devices of interest, can be developed.

A.1.2 An important class of biomaterials are resorbable polymers and copolymers based on lactic and glycolic acid. These polymers are either produced by a condensation reaction with or without any catalyst from the hydroxyacids (low molecular mass polymers), or by a catalyst-initiated ring-opening polymerization of the cyclic di-esters, lactide and glycolide.

A.1.3 Because lactic acid is optically active, the lactide exists in four different forms:

— the optically active L- and D-lactide,

— the racemic D,L-lactide, and

— the optically inactive meso-lactide.

A.1.4 Copolymerization of the different lactides with glycolide as a comonomer, leads to a number of various polymeric materials, which differ significantly in their overall properties.

In addition to glycolide, there are other monomers such as trimethylenecarbonate, dioxanone or caprolactone which can be homopolymerized and/or copolymerized with lactide or glycolide. Some products based on these materials are already on the market or under development.

A.1.5 Validated test specifications will be given for all resorbable polyesters based on lactic and glycolic acids, which are commercially available:

a) homopolymers:

— poly(L-lactide),
— poly(D,L-lactide),
— poly(glycolide);

b) copolymers:

— poly(L-lactide-co-D,L-lactide),
— poly(D,L-lactide-co-glycolide).

Finally, some remarks will be made on special analytical techniques, which are sometimes used to characterize these types of polymers.

A.2 Test specifications

A.2.1 Identity

The purpose is to ensure that the right polymer is under investigation.

Methods will include the following:

a) **^{1}H–NMR**. All polymers and copolymers show a different ^{1}H–NMR pattern. The polymer under investigation should match the comparison spectrum.

The procedure is as follows. Dissolve about 5 mg of the polymer in 0,5 ml deuterochloroform or another appropriate NMR solvent (dioxane, dimethyl sulfoxide, DMSO) under stirring with a small magnetic bar. Transfer the solution into a NMR tube and record the spectrum with integration.

Frequency: at least 250 MHz

Range: 5 000 Hz

Internal standard: TMS

b) **FTIR**

The polymer under investigation has to match the reference spectrum. Established techniques should be used for sample preparation.

A.2.2 Determination of comonomer ratio

The purpose is to ensure that the right copolymer is under investigation. Especially in the class of poly(D,L-lactide-co-glycolides) the comonomer ratio has a strong influence on the degradation behaviour.

The methods are as follows:

a) **^{1}H–NMR** (for copolymers made from L or D, lactide and glycolide)

The signals of the C–H (glycolide) and C–H or C–H_3 (lactide) protons are clearly separated.

The procedure is as in A.2.1 a).

Measure the height of the appropriate integrals with a ruler and calculate the ratio of the comonomers by taking into account the different number of protons.

b) **Optical rotation** (for copolymer made from L- and D,L-lactide)

It has been shown experimentally that the composition of the copolymers of optically active L- and optically inactive D,L-lactide units can be determined by the measurement of the optical rotation after a simple calibration with the pure homopolymers. That means that in the molecular mass range observed, the copolymers behave like a mixture of optically active and inactive compounds.

The procedure is as follows. Dissolve 1 g of the polymer in 200 ml dichloromethane and measure the specific optical rotation at 25 C (D-line, cell = 1 dm). Compare this value with a calibration curve, produced by mixing pure poly(L-lactide) of high optical purity with known amounts of pure poly(D,L-lactide) and note the composition.

Poly(L-lactide) of high optical purity should have specific optical rotation of at least 158° in dichloromethane at 25 °C. Usually there is a true linear relation between the optical rotation and the composition. Therefore only a few calibration points need be determined to check linearity.

A.2.3 Determination of residual monomers

Residual monomers have an impact on the stability of the polymers and can influence the processing of the material. Some monomers may also be harmful if present in excessive amounts.

Methods are as follows:

a) **GC** (for soluble polymers)

The procedure is as follows. The internal standard method is used with gamma-decalactone (> 99,5 %) as an internal standard.

Packed column GC system

Standard solution: Make a 10 ml solution of 80 mg lactide and 30 mg gamma-decalactone in dichloromethane.

Internal standard solution: Dissolve 15 mg gamma-decalactone in 10 ml dichloromethane.

Sample solution: Dissolve 50 mg polymer in about 7 ml dichloromethane, add 2 ml of the internal standard solution and fill up to 10 ml.

Inject standard and sample solution three times.

Chromatographic conditions:[2)]

GC: e.g. Hewlett-Packard 5790A with FI detector

Column: 1 m glass, I.D 2 mm

Stationary phase: 3 % OV-225 on Chromosorb W-HP 100/200 me

Injection volume: 1 µl

Injector: 180 °C

Detector: 300 °C

Column oven: 5 min at 100 °C, then with 5 °C/min to 185 °C

Carrier gas: Nitrogen, 30 ml/min

Burning gas: Hydrogen, 30 ml/min

Compressed air: 30 ml/min (analytical grade)

Capillary column GC system

Standard solution: Make a 10 ml solution of 80 mg lactide and 30 mg gamma-decalactone in chloroform.

2) Tradenames are indicated. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Internal standard solution: Dissolve 150 mg gamma-decalactone in 50 ml chloroform.

Sample solution: Dissolve 100 mg polymer in about 70 ml chloroform, add 2 ml of the internal standard solution and fill up to 100 ml.

Inject standard and sample solution three times.

Chromatographic conditions:

GC: e.g. Carlo Erba MEGA HRGC with FI detector

Column: 25 m fused silica, I.D. 0,32 mm

Stationary phase: DB-225 (film thickness 0,25 µm)

Injection volume: 1 µl

Split: 1:100

Injector: 80 °C

Detector: 300 °C

Column oven: 180 °C isotherm

Carrier gas: Hydrogen, 0,5 bar

Burning gas: Hydrogen, 0,6 bar

Compressed air: 1,1 bar (analytical grade)

Make up gas: Nitrogen, 1 bar

b) **^{1}H–NMR** (for soluble polymers)

Instead of GC ^{1}H–NMR (> 250 MHz) may also be used to determine the monomer content. However this method is not as accurate as the GC method and gives results which are usually too high.

The procedure is as in A.2.1 b).

Measure by means of a ruler the integrals of the monomer peaks (a doublet at about 1,7 ppm for the lactides, a singlet at about 4,9 ppm for the glycolide) and the integrals of the polymer peaks (a quartet or multiplet at about 5,2 ppm for the lactides, a multiplet at about 4,8 ppm for the glycolide; do not use the lactide polymer peak at about 1,6 ppm for these measurements, because this signal often interferes with the signal of traces of water) and calculate the content of residual monomer. For the copolymers take into account the different number of protons and the different relative molecular masses of lactide and glycolide.

c) **Method**: Extraction with a suitable solvent; determination of the extracted monomer by GC (for insoluble polymers)

Some modifications of polyglycolide are insoluble even in very polar solvents like hexafluoroisopropyl alcohol. To determine the residual glycolide, the polymer sample is extracted for a prolonged period of time with boiling ethylacetate. Since some oligomers are also extracted into ethylacetate, the glycolide content of the dried extract needs to be determined by GC.

A.2.4 Determination of residual solvents

Residual solvents may be present in the polymers due to production or purification processes. Some solvents may be harmful if present in excessive amounts. Solvents can also act as internal plasticizer and may influence the overall degradation behaviour.

GC headspace, external standard method

The procedure is as follows. Dissolve 200 mg polymer in a 10 ml vial with 2,0 ml 1,4-dioxane. Prepare an external standard by mixing appropriate amounts of the solvent to be determined in 1,4-dioxane.

Dissolve 200 mg polymer in a 10 ml vial with 2,0 ml of the external standard solution.

Prepare both solutions at least three times and run the chromatogram on a suitable GC headspace system.

Chromatographic conditions:

GC: e.g. HP 5792A with FI detector

Column: 2 m glass, I.D. 2 mm

Stationary phase: 5 % FFAP on Chromosorb G-AW-DMCS 80/100 mesh

Injector: 200 °C

Detector: 280 °C

Column oven: 145 °C

Carrier gas: Helium, 36 ml/min

Burning gas: Hydrogen, 30 ml/min

Compressed air: 30 ml/min (analytical grade)

Head space sampler

Thermostat the samples for 60 min at 70 °C to 80 °C.

Inject 250 µl of the vapour phase of both solutions. Repeat this step with the other vials and calculate the amount of residual solvent. The residual solvents in insoluble PGA have to be dissolved after hydrolysis in hot diluted sodium hydroxide.

A.2.5 Determination of tin content

Tin(II) salts are normally used as a catalyst for the ring opening polymerization of lactides and glycolides.

AAS method

The procedure is as follows. Dissolve about 200 mg of the polymer under gentle warming in 10 ml of a 20 % TBA (Tetrabutylammoniumhydroxide) solution.

Cool this solution and dilute to 20 ml.

Prepare a standard tin solution in the same concentration range.

AAS conditions:

Atomization: Graphite oven

Inert gas: Argon

Lamp: EDL

Wavelength: 224,6 nm

Slit: 0,7 nm

Background comp.: Deuterium lamp

The amount of tin is determined by the extrapolation method.

A.2.6 Determination of water content

Resorbable polyesters based on lactic and glycolic acid are degraded by simple hydrolysis. Although small amounts of water do not affect the polymer under proper storage conditions, high water contents may prematurely degrade the polymer.

The method used is the biamperometric Karl-Fischer titration process.

a) **Polymers, soluble in titration solvent**

The procedure is as follows. Prepare the solvent by mixing 50 ml 1,4-dioxane and 50 ml e.g. hydranal buffer. The solvent shall be made freshly before every determination and shall be titrated before use.

Dissolve 1 g of polymer in 100 ml solvent, wait 40 s and titrate with e.g. hydranal composite.

b) **Polymers, insoluble in titration solvent**

The procedure is as follows. Prepare the solvent by mixing chloroform and e.g. hydranal buffer in a 2:1 ratio. Weigh in 2 g of the polymer in a 100 ml volumetric flask and add the solvent to the calibration mark. Close the flask and stir for 48 h. Take 20 ml of the solution, wait 40 s and titrate it with e.g. hydranal composite.

Run a blank under the same conditions.

A.2.7 Determination of inherent viscosity

The viscosity of polymers soluble in the solvents listed below is directly related to the relative molecular mass average of the polymer. Nearly every polymer property is related to the molecular mass.

The method is by determination of the relative viscosity of a diluted polymer solution in an Ubbelohde type viscometer.

a) **Solvent chloroform**

The procedure is as follows. Dissolve 100 mg of polymer in a 100 ml volumetric flask. For a polymer with an expected inherent viscosity below 1,2 dl/g the measuring accuracy shall be 2 mg and for an expected inherent viscosity above 1,2 dl/g the measuring accuracy shall be 1 mg. Determine the relative viscosity of the polymer solution in the capillary of the viscometer at 25 °C ± 0,02 °C. For a polymer with an expected inherent viscosity below 1,2 dl/g the capillary of the viscometer shall be 0, and for a polymer above 1,2 dl/g the capillary shall be 0c. Correct the flow times according to the Hagenbach correction. Calculate the inherent viscosity by taking the natural logarithm of the relative viscosity and divide it by the concentration in g/dl.

b) **Solvent hexafluoroisopropanol (HFIP)**

The inherent viscosity in this solvent is determined at 30 °C ± 0,02 °C in a viscometer. In a volumetric flask, dissolve 50 mg ± 2 mg of polymer in HFIP. Dilute to 50 ml.

A.3 Special analytical techniques

A.3.1 Gel Permeation Chromatography (GPC), also called Size-Exclusion Chromatography (SEC), is widely used to determine the relative molecular mass distribution and to calculate molecular mass averages. It is a relative method which is calibrated against polymer standards. Narrow molecular mass distribution polystyrene or polymethylmethacrylate standards are widely used. Separation in GPC occurs by hydrodynamic volume of the swollen polymer in solution and not by the true molecular mass. Calibration with polymer standards works well only for those polymers that are closely related in chemical structure. The MW values for bioresorbable polymers from GPC may be too high because the more rigid backbone of the polyester gives rise to a bigger coil compared to polystyrene. The "universal calibration method" cannot be used to overcome these problems because there are no reliable Mark-Houwink parameters for selected polymers. Resorbable polymers show strong solvent and concentration dependence in GPC. Chloroform and tetrahydrofuran are the solvents of choice of polylactides and HFIP for polyglycolide. Tailing of the polymer peak can cause incorrect MW values.

The GPC method uses a Merck-Hitachi 655A-12 Liquid Chromatograph with pulse dumper, Rheodyne 7125 valve with a 100 loop attached, and an optional column oven at 30 °C, in-line 2 µm filter. The detector may, for example, be a ERMA ERC-7510 RI-detector, range 1 000 mV, peak height about 3 mV to 4 mV, temperature set to 35 °C.

Three GPC-columns (e.g. PL-Gel, PSS) of dimensions 300 mm × 7,5 mm with packing material of 10 µm and following exclusion limits:

Low MW set: 1 × E2, 1 × E3 and 1 × E5,

High MW set: 1 × E3, 1 × E5 and 1 × E6, may be used.

Mobile phase: Chloroform, degassed by vacuum filtering (membrane filter, 0,45 µm)

Flow: 1 ml/min

Backpressure: approximately 35 bar

Temperature: ambient, detector internal thermostat at 30 °C

Calibration: narrow relative molecular mass distribution polystyrene standards

Sample: about 25 mg to 50 mg polymer dissolved in 100 ml chloroform

The equipment is housed in a hood with weak evacuation to remove chloroform vapour. This also provides excellent temperature stability.

It has been reported that HFIP (hexafluoroisopropanol) may affect the stability of the seals and columns. Calibration in HFIP may be done against narrow relative molecular mass distribution polymethylmethacrylate standards.

A.3.2 Differential Scanning Calorimetry (DSC) is used to determine the glass transition, the recrystallization and the melting range of amorphous and semicrystalline polymers. Polymer blends can also be characterized with this technique.

Heating rates between 5 K/min and 20 K/min are common. To overcome the "thermal history" of a sample, measurements are often made with quenched samples. However care has to be taken that during such melting/cooling cycles, no thermal degradation of the polymer occurs.

A.3.3 High Resolution Nuclear Magnetic Resonance: high resolution $^{1}H-NMR$ and $^{13}C-NMR$ has been used to determine the configurational structures of lactic acid stereocopolymers. A quantitative $^{13}C-NMR$-based method can be used to determine the sequence of poly(D,L-lactide-co-glycolide). By monitoring the C = O signal of the glycolic acid part, the average chain length of the glycolic units can be determined. The method is sensitive for the distribution of the glycolic acid (GA-GA) and glycolic acid-lactic acid (GA-LA) dyads, when using deuterated DMSO as solvent.