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Université Abou BakrBelkaïd de Tlemcen

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Département de Génie Biomédical

MEMOIRE DE PROJET DE FIN D'ETUDES

Pourl'obtention du Diplôme de

MASTER en GENIE BIOMEDICAL

***Spécialité :*Imagerie Médicale / instrumentation biomédicale**

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USING X-RAYS IN BIOMATERIALS

Soutenu le14/09/ 2017 devant le Jury

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Année universitaire 2017-2018

ACKNOWLEDGEMENT

Thanks to "ALLAH" for the strength that he gives us that helped and supported us.This work has been realized in the frame ofERASMUS + Internationalstudent exchange program session February-July 2017 in LUBLIN UNIVERSITY OF TECHNOLOGY, POLAND.

We would like to thank all the stuff from both countries for all the hard work for trying to make everything easier and to create such a memorable experience.

Special thanks to:

Mr SalimKERAI, head of department of biomedical engineering, University Tlemcen.

MrSylwester SAMBORSKI, head of department of mechanical engineering, University Lublin.

MrFethiREGUIG BEREKSI and Abdel hafid BESSAID, Professors, Responsibles, University Tlemcen.

Mme Monika OSTAPIUK, Supervisor Professor and framer, University Lublin.

Mr Omar BEHDADA, Home University Coordinator, University Tlemcen.

Mme Anna RADZISZEWSKA, Host University Coordinator, University Lublin.

We would first like to thank our thesis advisor Mr SOULIMANESofianeand Mr DIB Nabil for accepting being examiners and juries members.

Finally, we must express our very profound gratitude to our parents and family for providing us with unfailing support and continuous encouragement throughout our years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

OUTLINES:

I. CHAPTER I: INTODUCING BIOIMATERIALS

1. **INTRODUCTION**
2. **BIOMATERIALS DEFINITION**
3. **HISTORY**
4. **BIOMATERIALS USES**
5. **LIST OF BIOMATERIALS**
6. **CHARACTERISTICS OF BIOMATERIALS**
7. **BIOCOMPATIBILITY**

II. CHAPTER II: INTODUCING X-RAYS

1. **INTRODUCTION**
2. **WHAT ARE X-RAYS?**
3. **X-RAYS SOURCES: HOW ARE X-RAYS PRODUCED?**
4. **X-RAYS TUBES**
5. **THE X-RAYS SPECTRUM**
6. **RADIOACTIVE SOURCES**
7. **THE USE OF X-RAYS**
8. **CONCLUSION**

III. CHAPTER III: USING X-RAYS IN BIOMATERIALS

IV. X-RAY PHOTOELECTRON SPECTROSCOPY

a. **INTRODUCTION**

b. **DIFFENITION**

c. **PRINCIPE**

3.1. INSTRUMENTATION

3.2 MEASUREMENT

d. **ADVANTAGES / DISADVANTAGES**

GENERAL INTRODUCTION:

The study of biological and biomaterials with X-rays provides an important contribution to the understanding of the structure-function relationship in natural and artificial materials.

Biomaterial is used to be devices to replace a part or a function of the body in a safe, reliable, economic and physiologically acceptable manner.

Researchers in the fields of medicine, biology and engineering are investigating new techniques for the use of materials in the human body. These approaches are designed to help body parts to restore its movement and vitality.

Aside from the fundamental knowledge gained by these insights which is of value already itself it helps to create new materials which are either bio-inspired or modified such that they can interact in an optimized way with the biological environment.

While the specifics of a given strategy may vary, an approach typically involves some combination of biomaterials which we will try to find its characteristics using X-rays.

In this work we present three chapters; in chapter one, we will try to talk about biomaterials in theory to have an idea about what it really is. In chapter two we introduce famous X-rays then we added some applications in chapter three. In chapter three we will talk about techniques based in x-rays to identify and characterize biomaterials.

CHAPTERI:

INTODUCING BIOIMATERIALS

CHAPTER 1: INTRODUCING BIOMATERIALS

OUTLINE:

1. INTRODUCTION
2. BIOMATERIALS DEFINITION
3. HISTORY
4. BIOMATERIALSUSES
5. LIST OF BIOMATERIALS
6. CHARACTERISTICS OF BIOMATERIALS
7. BIOCOMPATIBILITY

CHAPTER 1: INTRODUCING BIOMATERIALS

1. INTRODUCTION:

In this chapter we will have an overview on Biomaterials. What are they? Some history, it uses and functions, and some characteristics finally we will mention few applications.

2. BIOMATERIALS DEFINITION:

A biomaterial is any substance natural or synthetic material (such as a metal or polymer) that has been engineered ,being suitable and biocompatible to interact with living tissue or biological systems for a medical purpose especially as part of a medical device - either a therapeutic (treat, augment, repair or replace a tissue function of the body) or a diagnostic one. As a science, biomaterials are about fifty years old. The study of biomaterials is called biomaterials science or biomaterials engineering. It has experienced steady and strong growth over its history, with many companies investing large amounts of money into the development of new products. Biomaterials science encompasses elements of medicine, biology, chemistry, tissue engineering and materials science.

Note that a biomaterial is different from a biological material, such as bone, that is produced by a biological system. Additionally, care should be exercised in defining a biomaterial as biocompatible, since it is application-specific. A biomaterial that is biocompatible or suitable for one application may not be biocompatible in another.

3. HISTORY:

- More than 2000 years ago, Romans and Chinese used gold in dentistry.
- 1937 Poly (methyl methacrylate) (PAMMA) introduced in dentistry.
- 1958 Rob suggests Dacron Fabrics can be used to fabricate an arterial prosthetic.
- 1960 Charnley uses PAMMA, ultrahigh-molecular-weight polythylend, and stainless steal for total hip replacement.
- Late 1960 – early 1970’s biomaterial field solidified.
- 1975 Society for Biomaterials formed.

4. BIOMATERIALS ARE USED IN:

Biomaterials can be derived either from nature or synthesized in the laboratory using a variety of chemical approaches utilizing metallic components, polymers, ceramics or composite materials. They are often used and/or adapted for a medical application, and thus comprise whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function. Such functions may be relatively passive, like being used for a heart valve, or may be bioactive with a more interactive functionality such as hydroxyl-apatite coated hip implants. Biomaterials are also used every day in dental applications, surgery, and drug delivery. For example

- Joint replacements
- Bone plates
- Bone cement
- Artificial ligaments and tendons
- Dental implants for tooth fixation
- Blood vessel prostheses
- Heart valves
- Skin repair devices (artificial tissue)
- Cochlear replacements
- Contact lenses
- Breast implants
- Drug delivery-mechanisms
- Sustainable materials
- Vascular grafts
- Stents
- Nerve conduits
- Surgical sutures, clips, and staples for wound closure
- Pins and screws for fracture stabilization
- Surgical-mesh

5. LIST OF BIOMATERIALS:

0–9
- 3D bio-printing

A
- Abducting
- Alacrite
- Algaenan
- Alloderm
- Aluminum oxide

B
- Richard Berry (scientist)
- Bio-ink
- Bioabsorbablemetallic glass
- Bioactive glass
- Bio ceramic
- Biocompatibility
- Bio glass
- Biomesh
- Biopolymer
- Bioresorbablemetal
- Bonecement
- Bone wax

C
- Cell encapsulation
- Cobalt-chrome
- Coax (material)
- Co-polyester

E
- Evocative Design
- Elastin

F
- Fibrin scaffold
- Fluorosilicate glass

H
- Havar (alloy)
- Hyalobarrier
- Hydroxyapatite

M
- Magnetically assisted slip casting
- Biomimetic material
- Mechanicalproperties of biomaterials
- Medical grade silicone
- Medical uses of silver
- Metalfoam
- Metals in medicine
- Miniature organs
- Mainsheet perfusion culture system

N
- Nano cellulose
- Nano topography
- Nickel titanium

O
- Oxinium

P
- Poly(methyl methacrylate)
- Polyanhydrides
- Polydimethylsiloxane
- Polydioxanone
- Polyethylene glycol
- Polyethyleneterephthalate
- Polyglycolide
- Polyhydroxyalkanoates
- Polyimide
- Polytetrafluoroethylene
- Polyvinylidenefluoride

S
- Self-healing material
- Shrilk
- Silk
- Star lite
- Surface and bulk erosion
- Surface chemistry of neural implants
- Surface modification of biomaterials with proteins
- Surgicalstainlesssteel
- Synthesis of bio glass
- Syntheticbiodegradablepolymer

T
- Tantalum
- Thermoplastic elastomer
- Ti-6Al-7Nb
- Titanium
- Titaniumbiocompatibility
- Tricalcium phosphate

V
- Vitallium

Z
- Zirconium dioxide

6. CHARACTERISTICS OF BIOMATERIALS:

Physical Requirements

- Hard Materials.
- Flexible Material.

Chemical Requirements

- Must not react with any tissue in the body.
- Must be non-toxic to the body.
- Long-term replacement must not be biodegradable.

7. COMPATIBILITY:

Biocompatibility is related to the behavior of biomaterials in various environments under various chemical and physical conditions. The term may refer to specific properties of a material without specifying where or how the material is to be used. For example, a material may elicit little or no immune response in a given organism, and may or may not able to integrate with a particular cell type or tissue. The ambiguity of the term reflects the ongoing development of insights into how biomaterials interact with the human body and eventually how those interactions determine the clinical success of a medical device (such as pacemaker or hip replacement). Modern medical devices and prostheses are often made of more than one material—so it might not always be sufficient to talk about the biocompatibility of a specific material.

Physiological state of mutual co-existence between a biomaterial and the environment such as either has an undesirable effect on the other. Or it also means that materials described display good or harmonious behavior in contact with tissue and body fluid.

BIOINERT: No host response to the material

BIO FUNCTIONALITY: Playing a specific function in physical and mechanical terms.

Conclusion:

The importance of biomaterial in medical field obligates the researchers to find other techniques to know what to and how to characterize biomaterials. So in this thesis we try to use new techniques based on x-rays .what the characteristics of x-rays that make it usable in chemical analysis?

CHAPTERΠ:

INTODUCING

X-RAYS

OUTLINE:

1. **INTRODUCTION**
2. **WHAT ARE X-RAYS?**
3. **X-RAYS SOURCES: HOW ARE X-RAYS PRODUCED?**
4. **X-RAYS TUBES**
5. **THE X-RAYS SPECTRUM**
6. **RADIOACTIVE SOURCES**
7. **THE USE OF X-RAYS**
8. **CONCLUSION**

1. INTRODUCTION:

One of the most discoveries in the world that changed the human historyis X-rays. X-rays were discovered in 1895 by Wilhelm Conrad Roentgen (1845-1923). They are electromagnetic radiation with wavelengths of the order of 10-10 m. This corresponds to photon energies in the kilo electron volt region. So what are the characteristics of X-rays that make them useful in different fields? How can they be detected?

In this chapter we will talk about x-ray definition, sources and characteristics.

2. WHAT ARE X-RAYS?

X rays are a kind of super-powerful version of ordinary light: a higher-energy form of electromagnetic radiation that travel at the speed of light in straight lines (just like light waves do)..That means their frequency (how often they wiggle about) is correspondingly greater. And, because the energy of electromagnetic waves is directly related to their frequency, X rays are much more energetic and penetrating than light waves as well.

3. X-RAYS SOURCES:HOW ARE X-RAYS PRODUCED?

X-rays are produced by the interaction of electrons with a metal target (see Diagram). The electrons are emitted by a filament heated by Joule effect (thermal electrons). These electrons are accelerated by a potential difference and directed towards a metal target (anode or anticathode). The production of photons X is due to the rapid deceleration of the electrons during their impact on the target. Note that the yield of production of the X-rays is low, typically of the order of 0.2%; the rest of the energy dissipates as heat. It is therefore necessary to evacuate this heat (the need for a cooling system) and to use good thermal conductive target materials with high melting points (refractory metals: tungsten, molybdenum or very good conductors: copper).

4. X-RAYS TUBES:

The process takes place in vacuum X-ray tubes, also called "Coolidge tubes". X-rays come out of the tube through beryllium windows, a material selected for its vacuum seal and for its X-ray permeability. However, the production of X-rays by this means does not show an efficiency of more than 1%!

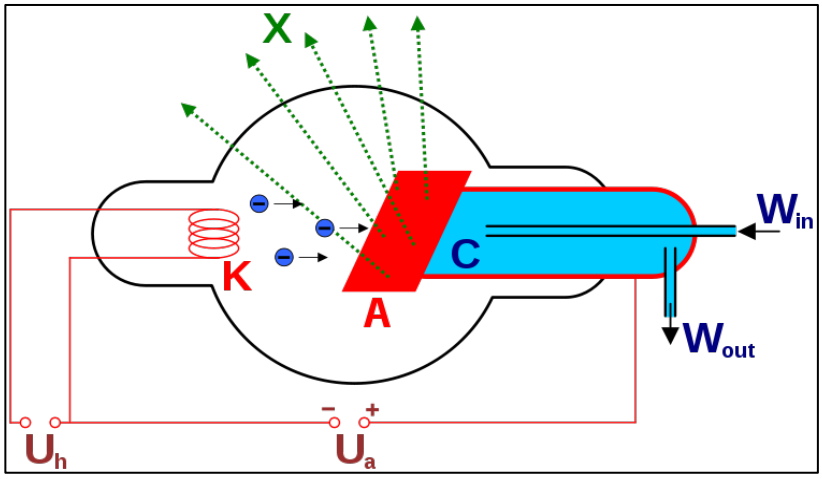


Figure: x-ray tubes

5. THE X-RAYS SPECTRUM:

The X-ray emission spectrum consists of the superposition of a continuous spectrum and a discrete spectrum of lines. X-rays are in fact derived from the rapid deceleration of electrons during impact on the target (braking radiation or Bremsstrahlung) or the return to the ground state of excited atoms of the target.

6. RADIOACTIVE SOURCES:

The radioactive decay of certain isotopes also produces X-rays. This production is made from compact sources, but it is difficult to control and is not stable.

7. THE USE OF X-RAYS:

For many years ago, the applications of X-rays were essentially medical, whether imaging or therapy. As to their origin, throughout this period, it remained highly controversial. In 1912, Max von Laue predicted that X-rays should be diffracted by crystals. Thanks to this method, it became possible to obtain the position of the atoms in the solids, and therefore to describe its structure. The first resolved structure was that of the sodium chloride by Sir William Henry Bragg and his son. The end of the 1940s was marked by three important events:

For the first time, radiation emitted by relativistic electrons (whose velocity is close to that of light) was observed, accelerated by means of magnetic fields in machines (synchrotrons) built for the study of the physics of elementary particles . This radiation was called synchrotron radiation.

The X-rays began to determine the first protein structures, complex biological systems consisting of several tens of thousands of atoms.

8. CONCLUSION:

The x-rays are surpassed their unique use in medical imaging and they are used for the analysis of biomaterials thanks to their advantages and importance which made them usable in different fields using a lot of techniques based on x-ray .

CHAPTERIII:

USING X-RAYS IN BIOMATERIALS

OUTLINE:

I. USING X-RAY MICRO COMPUTED TOMOGRAPHY TO VISUALISE BIOMATERIALS

1. **X-RAY COMPUTED TOMOGRAPHY (CT):**
2. **NANO-TOM®:**
3. **PRINCIPLE OF OPERATION**
4. **STAGES OF IMAGE ACQUISITION**
5. **PRINCIPLE OF CT: RECONSTRUCTION METHOD**
6. **APPLICATIONS**
7. **KEY PRACTICAL ISSUES**
8. **LIMITATIONS**
9. **CURRENT STATE OF THE ART**
10. **CONCLUSIONS:**

II. RADIOPAQUE IODINATED POLYMERIC NANOPARTICLES FOR X-RAY IMAGING APPLICATIONS

1. **PREPARATION OF RADIOPAQUE POLYMERS:**
2. **EXPERIMENTAL**

   2.1.MATERIALS

   2.2.METHODS

   2.2.1. SYNTHESIS OF MAOETIB

   2.2.2. ***SYNTHESIS OF polyMAOETIBNANOPARTICLES***

   2.2.3. IN VIVO IMAGING

   2.3.CHARACTERIZATION
3. **RESULTS AND DISCUSSION**
4. **CONCLUSIONS**

III. X-RAY PHOTOELECTRON SPECTROSCOPY

1. **INTRODUCTION**
2. **DIFFENITION**
3. **PRINCIPE**

3.1. INSTRUMENTATION

3.2 MEASUREMENT

4. **ADVANTAGES / DISADVANTAGES**

I. USING X-RAY MICRO COMPUTED TOMOGRAPHY TO VISUALISEBIOMATERIALS:

1. X-RAY COMPUTED TOMOGRAPHY (CT):

- CT is non-destructive technique for visualizing solid interiors
- X-ray attenuation is a function of density of the material
- Images are created by X-rays in multiple orientations
- A specialized algorithm reconstructs the distribution of X-ray attenuation in the slice plane.

2. MAINBENEFITS:

- 3-D examination
- Increased speed of data acquisition
- Internal visualisation without destruction

3. SCALES OF OBSERVATIONIS KEV:

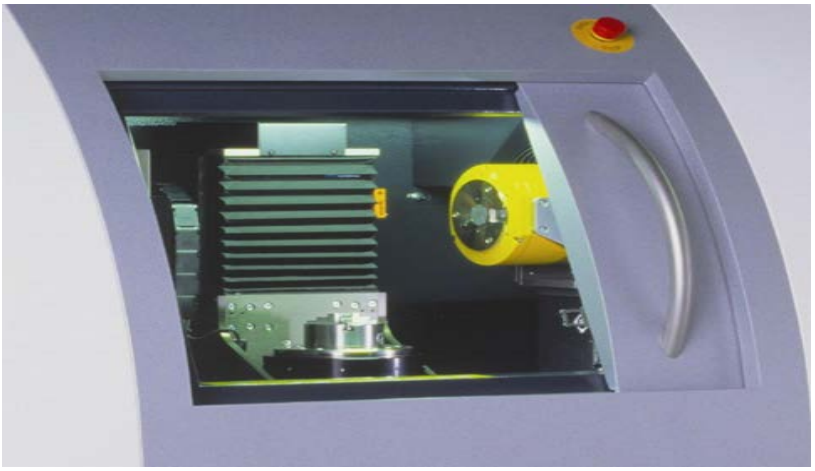
- Medical (Macro-scale) scanner >500 µm (max sample = human body)
- Research (Micro-scale) scanner ~ 0.5-500 µm (max sample = drinks can)

4. NANO-TOM®: ultra-high resolution, Nano CT system

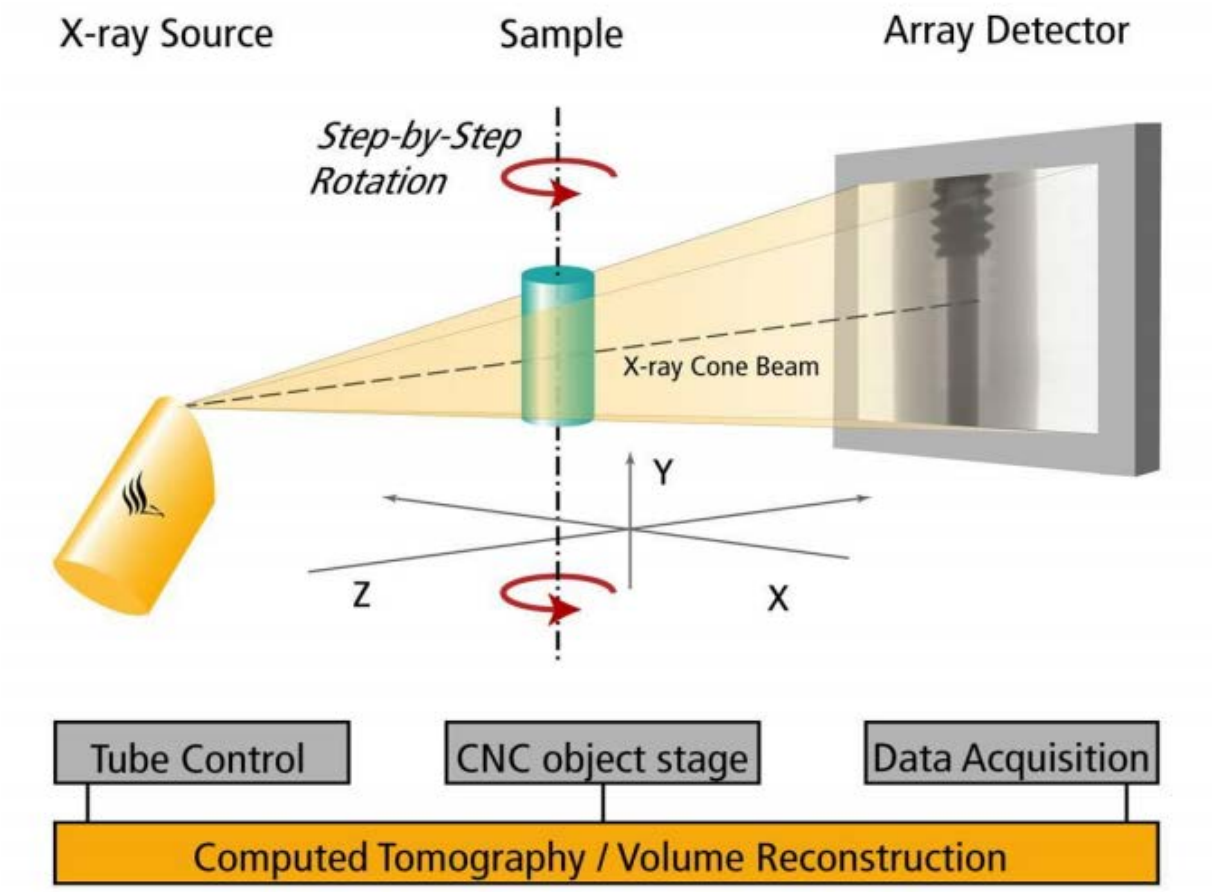
X-RAY TUBE: Nano focus < 800 nm spot size / 180 kV / 15 W

X-RAYDETECTOR: Flat panel, 5 M-pixel,15 M-pixel virtual detector, 50 µm pixel sizes

MANIPULATOR:5 axis stepper motors, granite-based, high-precision air bearing



5. PRINCIPLE OF OPERATION:



6. STAGESOF IMAGE ACQUISITION

STAGE ONE

- Prepare & Position Sample
- Choose Scanning Energy & Resolution
- Correct for Artefacts
- Scan

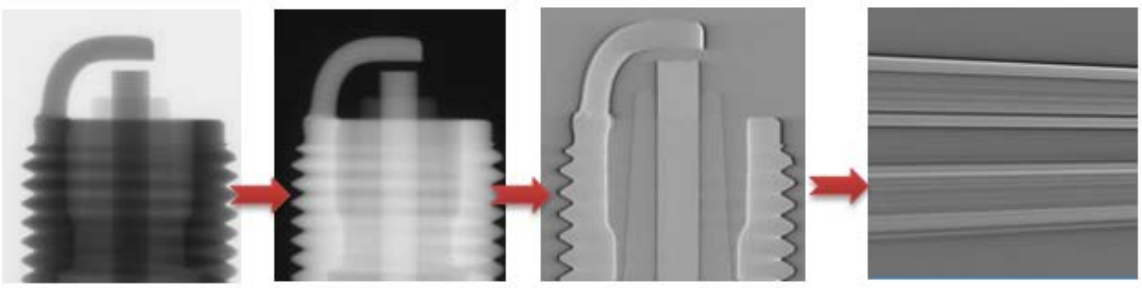
STAGE TWO

- Open Projection Images in Recon Software
- Correct for Artefacts
- Reconstruct

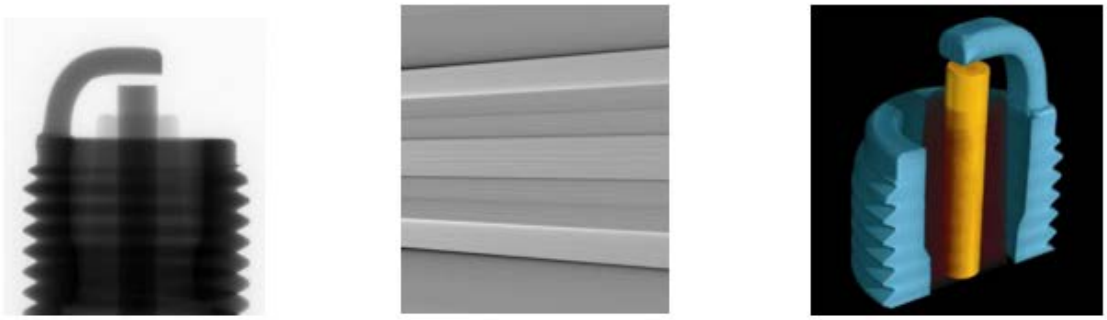
- Visualise

7. PRINCIPLE OF CT: RECONSTRUCTION METHOD

Example:spark plug



PROJECTION INVERSION LOG+FILTER BACK-PROJECTION



ACQUISITION OF 600 PROJECTIONS 600 BACK PROJECTIONS 3D VISUALIZATION

8. APPLICATIONS:

- The lower the voltage → the higher the contrast
- The higher the current → the lower the noise
- The higher the power → the lower the resolution

For each specimen type optimisation is necessary

9. KEY PRACTICAL ISSUES

- Max. Sample size varies between manufacturers
- Sample size and resolution are interlinked (although you can zoom in)
- Highest resolution ≠ best quality images
- Higher the material density, the higher the X-ray energy needed to penetrate the sample
- Increasing the projections improves image quality but increases scan time
- Without the best hardware, the process can be long…

10. LIMITATIONS:

• Better scans if the entire of object is in field of view at all projection angles.

• The object must be penetrated by X-rays at all projection angles - precludes long paths of high density material.

• Resolution is limited by:

- X-ray focal spot size and beam geometry;
- mechanical precision of object manipulator;
- detector resolution and sensitivity;
- Accuracy of centre of rotation.

11. CURRENT STATE OF THE ART

1. Improved Focus & Resolution
2. Higher Quality Detectors
3. Dealing with Artefacts
4. Major Computational Improvements

12. CONCLUSIONS:

- Currently the best method for non-destructive 3D imaging (certainly for multi-phase systems)
- Does not change internal structure
- No sample preparation necessary (and therefore no influence on structure)
- Visualisation of the internal structure, not restricted to surface
- Objects can be imaged during interaction with liquid (or gas?)
- Recent advances enable rapid, high resolution, high quality, 3D imaging in minutes

II. RADIOPAQUE IODINATED POLYMERIC NANOPARTICLES FOR X-RAY IMAGING APPLICATIONS:

Recent literature concerning novel biomaterials indicates increasing interest in developing radiopaque polymers as contrast agents for X-ray imaging. The radiopaque polymeric agents may be used for various applications, e.g. imaging of blood pool or certain body organs, in order to detect or diagnose various disease states, monitoring embolization processes, construction of implants used in surgery to determinate their exact location, and dental composition.

1. PREPARATION OF RADIOPAQUE POLYMERS:

There are different reported techniques describing the preparation of radiopaque polymers of various types. For example, radiopaque polymer blends have been prepared by incorporating radiopacifying agents such as heavy metal powders, inorganic salts of a heavy element, or organic compounds containing a heavy atom substituent as physical mixtures with an appropriate polymer.

Radiopaque polymer-salt complexes have been produced by incorporation of radiopaque heavy metal salts into an appropriate polymer ligand via chelation. Radiopaque polymers have also been formed by the polymerization of methyl methacrylate with metal salts of vinyl monomers such as barium or zinc acrylates, or by grafting iodine-containing molecules on to preformed high molecular weight polymers.

Another approach for preparing radiopaque polymers is based on homo- or co-polymerization of aromatic iodine containing vinylic monomers, such as triodophenyl methacrylate, 2-methacryloyloxyethyl(2,3,5-triiodobenzoate) [MAOETIB], 2-hydroxy-3-methacryloyloxypropyl(2,3,5-triiodobenzoate) and 3-(methacryloylamidoacetamido)-2,4,6-triiodobenzoic acid, with other vinylic monomers such as 2-hydroxyethyl metacrylate (HEMA) or methyl methacrylate (MMA) .

Radiopaque iodinated copolymericmicrospherical particles of relatively broad size distribution have been prepared by precipitation of iodinated copolymeric chains, or by

suspension polymerization of aromatic iodinated vinylic monomers with HEMA or ethylene glycol dimethacrylate . Recently, novel radiopaque homopolymeric and composite iodinated micron-sized spherical particles of narrow size distribution and high iodine content were prepared by Galperin and Margel by dispersion polymerization, encapsulation and swelling based techniques.

Radiopaque micron-sized particles can be used for X-ray imaging needs such as embolization, implants and prosthesis, but not for blood pool and body organ imaging, because of the danger of plugging blood vessels. For these purposes nanometer-sized particles are essential. However, these kind of radiopaque nanoparticles are not yet available for clinical use.

Recently, radiopaque magnetic γ-Fe_2O_3/polyMAOETIB core-shell nanoparticles of 15.0±2.4 nm were prepared by Galperin and Margel. However, the radiopacity of these nanoparticles is relatively low, since the γ-Fe_2O_3 core is scarcely radiopaque and the iodine content of these nanoparticles is only 23.9%.

The present article describes the synthesis of iodinated radiopaque polymeric nanoparticles of sizes ranging between 30 and 350 nm, by emulsion polymerization of the monomer MAOETIB, in the presence of sodium dodecyl sulfate (SDS) as surfactant and potassium persulfate (PPS) as initiator. The influence of various polymerization parameters, e.g., MAOETIB, PPS and SDS concentrations, on the molecular weight, polymerization yield, size and size distribution of the particles was elucidated. These polymeric nanoparticles composed of ca. 58% by weight iodine; therefore they expect to possess a significant radiopaque nature. In vitro radiopacity of the iodinated nanoparticles of 30.6±5.0 nm diameter dispersed in water and in the dry state was demonstrated with a CT scanner. In vivo CT-imaging performed in a dog model by intravenous administration of the uniform 30.6±5.0 nm diameter radiopaque nanoparticles dispersed in saline demonstrated significant enhancement of lymph nodes, liver, kidney and spleen.

2. EXPERIMENTAL

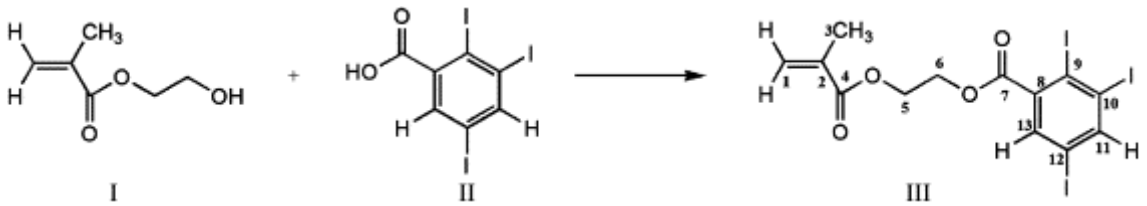
2.1.MATERIALS:

The following analytical-grade chemicals were purchased from commercial sources and were used without further purification:2,3,5-triiodobenzoic acid (Aldrich, 98%), HEMA (Aldrich 99%), 1,3-dicyclohexylcarbodiimide (DCC, Aldrich 99%), 4-pyrrolidinopyridine (Aldrich, 98%), diethyl ether anhydrous (Aldrich 99.7%), ethyl acetate (99.5%), PPS (Aldrich, 99+%), SDS (BHD 90%), toluene (Bio-Lab, HPLC grade). Water was purified by passing deionized water through an Elgastat Spectrum reverse osmosis system (Elga Ltd., High Wycombe, UK).

2.2. METHODS:

2.2.1. SYNTHESIS OF MAOETIB

The iodinated monomer MAOETIB was synthesized according to Scheme 1, as described in the literature. Briefly, 2,3,5-triiodobenzoic acid, II, (50 g, 0.10 mol), HEMA, I, (15 g, 0.11 mol), DCC (23 g, 0.11 mol) and 4-pyrrilidinopyridine (1.5 g, 0.010 mol) were dispersed in ether (500 mL), and then stirred at room temperature for 18 h. The formed solid was filtered off, and the residue washed with fresh ether. The ether solution was washed with HCl (2 N), saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$, filtered and evaporated to produce an orange solid. Pure white crystals of MAOETIB, III, (m.p. 95 °C) were obtained by recrystallization of the orange solid twice from ethyl acetate (yield 84%).



Scheme 1. Synthesis of MAOETIB.

^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H, CH_3), 4.57 and 4.48 (m, 4H, OCH_2CH_2O), 5.61 (s, 1H, olefinic), 6.16 (s, 1H, olefinic), 7.33 (d, 1H, *J*=1.68 Hz, Ar–H), 8.30 (d, 1H, *J*=1.68 Hz, Ar–H). ^{13}C NMR ($CDCl_3$) δ 18.33 (C-3), 61.92 (C-5), 63.93 (C-6), 93.64 (C-12), 106.56 (C-9),

113.39 (C-10), 126.41 (C-1), 135.72 (C-2), 137.13 (C-13), 148.86 (C-11), 165.60 (C-4), 166.97 (C-7). MS (ES^+): *m*/*z* 635 (MNa^+, 100). Elemental analysis (calculated): C, 25.52; H, 1.81; O, 10.46; I, 62.21. Found: C, 25.65; H, 1.82; O, 10.49; I, 62.04.

2.2.2. *SYNTHESIS OF polyMAOETIBNANOPARTICLES:*

PolyMAOETIB nanoparticles of 30.6±5.0 nm were prepared by emulsion polymerization of MAOETIB according the following procedure: 5 mL of toluene solution containing 400 mg MAOETIB ($^{2\%\ w/v_{H_2O}}$) were introduced into a vial containing 20 mL of 1% ($^{2\%\ w/v_{H_2O}}$) SDS aqueous solution and 0.05% ($^{2\%\ w/v_{H_2O}}$) PPS. The mixture was than shaken at 73 °C for 12 h. The organic phase containing the toluene and excess monomer was then extracted from the aqueous phase. Excess SDS was then removed from the aqueous dispersion by extensive dialysis against water. Dried radiopaque polyMAOETIB nanoparticles were then obtained by lyophilization. The effect of various polymerization parameters, such as monomer, initiator and surfactant concentrations, on the molecular weight, polymerization yield, size and size distribution of the particles has been elucidated.

2.2.3. IN VIVO IMAGING:

A dog model was used in order to evaluate the in vivo contrast ability of the polyMAOETIB nanoparticles. Saline dispersion of the polyMAOETIB nanoparticles of 30.6±5.0 nm (concentration 3 mg/mL; dose 40 mg/kg) was intravenously infused to one dog during 30 min (weight ca. 20 kg). Pelvic, abdominal and leg CT scans were performed before and 24 h after administration of the radiopaque nanoparticles. In addition, full blood tests including complete blood count and a full chemical analysis were performed before, 24 h and a month post nanoparticles injection. The dog was observed for 6 months post injection for any health changes.

2.3. CHARACTERIZATION:

^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX-300 spectrometer. Chloroform-*d* and tetrahydrofuran-d_8 (THF-d_8) chemical shifts are expressed in ppm downfield from tetramethylsilane used as internal standard.

Mass spectra were obtained with a Finnigan 4021 spectrometer (electrospray and desorption chemical ionization).

Elemental analysis was performed with an elemental analysis instrument (model EA1110, CE Instruments).

Iodine analysis was performed by the Microanalysis Lab., Institute of Chemistry, The Hebrew University of Jerusalem, JERUSALEM. The reported values are an average of measurements performed on at least three samples of each of the tested particles, and have a maximum error of about 2%.

Fourier Transform Infrared (FTIR) analysis was performed with a Bomem FTIR spectrophotometer; model MB100, Hartman & Braun. The analysis was performed with 13 mm KBr pellets that contained 2 mg of the examined particles and 198 mg KBr. The pellets were scanned over 200 scans at a 4 cm^{-1} resolution.
TEM pictures were obtained with a JEOL-JEM 100SX electron microscope with 80–100 kV accelerating voltage. Samples for TEM were prepared by placing a drop of diluted sample on a 400-mesh carbon-coated copper grid. The dry particles' average size and distribution were determined by measuring the diameter of more than 100 particles with the image analysis software AnalySIS Auto (Soft Imaging System GmbH, Germany).

The hydrodynamic diameter and size distribution of the nanoparticles dispersed in aqueous phase were determined using a sub-micron particle analyzer**,** model N4 Plus, Coulter Electronics Ltd., England.
The thermal behavior of the particles was measured by thermogravimetric analysis (TGA) and differential scanning calorimetric (DSC). The analysis was performed with a TC15 system equipped with TGA (model TG-50) and DSC (model DSC-30, Mettler–Toledo). The analysis was performed with approximately 10 mg of dried samples in a dynamic nitrogen atmosphere (200 mL/min) at a heating rate of 10 °C/min.

The molecular weight of polyMAOETIB was measured with a gel permeation chromatograph consisting of an Applied Bioscience 759A UV detector at 254 nm. The samples were eluted with dimethylformamide through a linear Styragel column (104 Å pore size) at a flow rate of 1 mL/min. The molecular weights were determined with respect to polystyrene standards with a Winer/286 computer program.

The radiopacity of the dried and water-dispersed polyMAOETIB nanoparticles was demonstrated by a CT scanner (MARCONI, HeliCAT II). The X-ray tube voltage of the CT apparatus is 150 kV. Quantization (in Hounsfield units (HU)) of the opacification of the nanoparticles was performed by image-processing software “CDP DiagNet v. 5.55”.

3. RESULTS AND DISCUSSION:

3.1. CACTERIZATION OF THE polyMAOETIBNANOPARTICLES:

Fig. 1A presents a typical TEM picture of the dried polyMAOETIB nanoparticles, prepared according to the experimental section [$2\%\ (w/v_{H_2O})$ monomer]. These iodinated radiopaque polymeric nanoparticles, after evaporation of the aqueous medium (required for preparation of the samples for electronic microscope analysis), form agglomerates. Despite this, it is easy to visualize that those agglomerates are composed of spherical nanoparticles with average diameter of 28.9±6.3 nm. On the other hand, polyMAOETIB nanoparticles dispersed in water are stable and possess a single population with average hydrodynamic diameter of 30.6±5.0 nm, as measured by light scattering technique (Fig. 1B). We can see therefore an excellent correlation between the dry and the hydrodynamic diameter of the nanoparticles, as measured by TEM and light scattering, respectively. The polyMAOETIB nanoparticles were free of traces of the monomer, as was verified by FTIR, by the lack of the C=C double-bond stretching band at about 1623 cm^{-1}, and by ^1H NMR (THF-d_8), by the lack of the 2 peaks of the vinylic protons at 5.61 and 6.16 ppm. It should also be noted that the polyMAOETIB nanoparticles contain ca. 58% iodine while the monomer contains 62%, as was measured by iodine elemental analysis. This difference in the iodine content may be due to the initiator fractions of the polymer and to the adsorption of the surfactant SDS on the surface of the polyMAOETIB nanoparticles. It is also possible that a few aromatic C–I bonds were cleaved during the radical polymerization of MAOETIB, leading thereby to a slight decrease in the iodine content of the polymer relative to the monomer. Fig. 2 presents TGA and DSC thermograms of the polyMAOETIB nanoparticles. It should be noted that no change in weight and voltage of the sample were observed between room temperature to approximately 270 °C. Fig. 2A indicates almost 100% weight loss at temperatures between 270 and 390 °C. This weight loss is due to the decomposition of the polyMAOETIB, as indicated by the endothermic DSC peak (Fig. 2B) at 352 °C. The dominant exothermic peak at 360 °C, following the endothermic peak at 352 °C, is due to the reaction between two iodine atoms (formed during the decomposition of polyMAOETIB) to produce I_2, as

confirmed by mass spectrometry (by the decreasing parent peak of the iodine atom and the increasing parent peak of I_2).

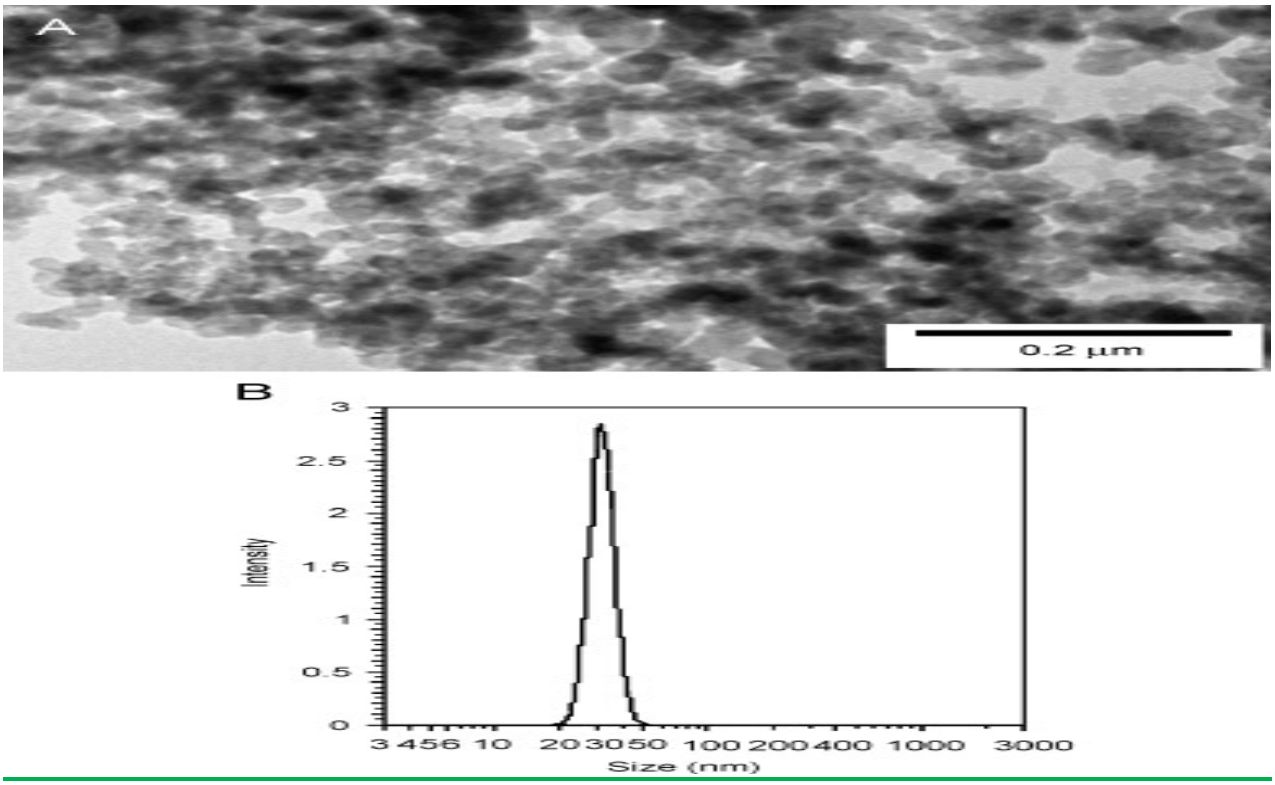


Fig. 1.

TEM photomicrograph (A) and size histogram (B) of the polyMAOETIB nanoparticles.

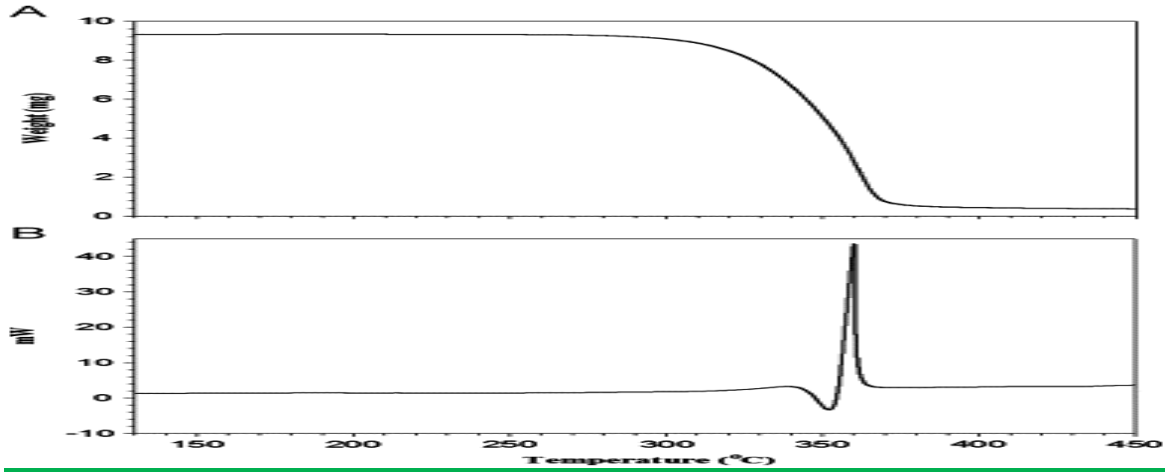


Fig. 2.

TGA (A) and DSC (B) of the 30.6±5.0 nm polyMAOETIB nanoparticles.

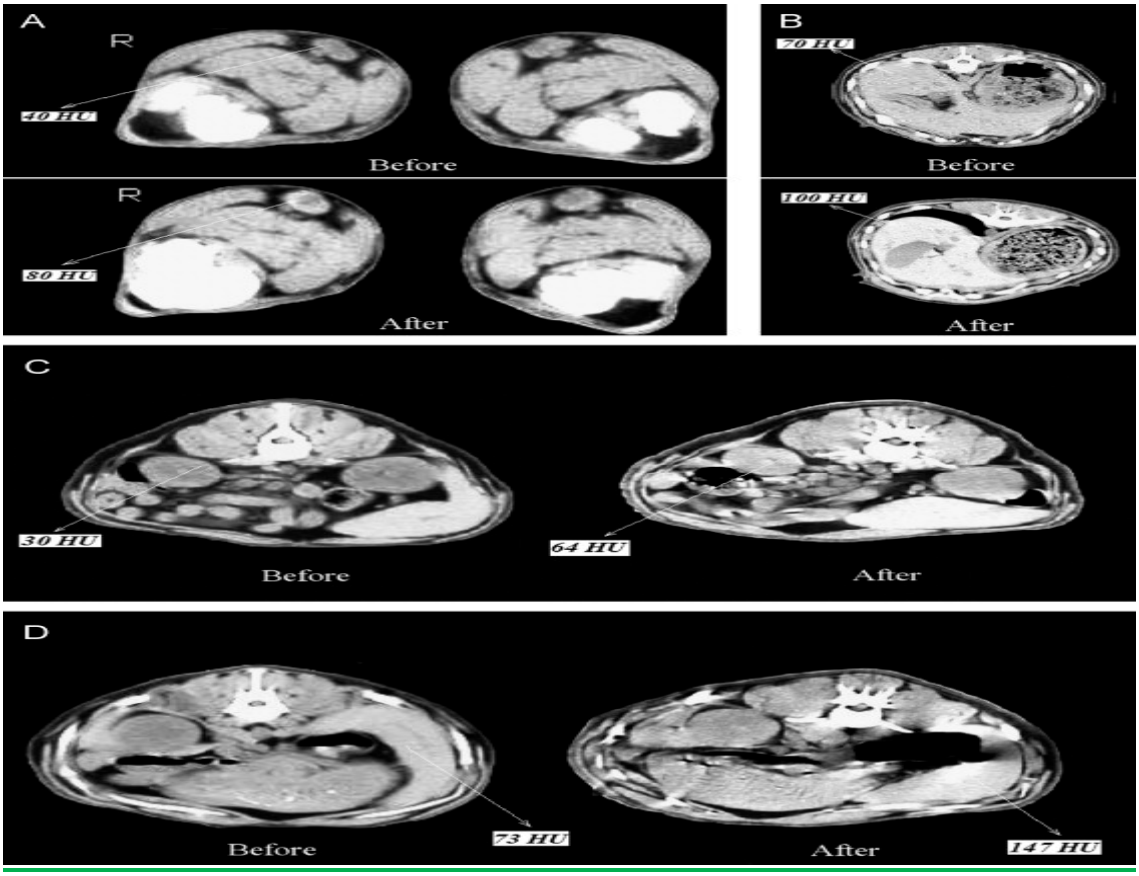


Fig. 6.

CT images and opacification (in HU) of various dog organs before and after 24 h intravenous administration of the 30.6±5.0 nm polyMAOETIB nanoparticles dispersed in saline: A—popliteal lymph nodes; B—liver; C—kidney; D—spleen.

4. CONCLUSIONS:

This part describes the synthesis and characterization of new iodinated radiopaque polymeric nanoparticles of 30.6±5.0 nm diameter formed by emulsion polymerization of MAOETIB. The effects of various polymerization parameters, e.g., monomer, initiator and surfactant concentrations, on the polymerization yield, molecular weight, size, and size distribution of the particles have been elucidated. In vitro and in vivo X-ray contrast properties of the polyMAOETIB nanoparticles illustrated that indeed these iodinated nanoparticles may be efficient candidates for being used as contrast agents in such important and clinically significant diagnostic imaging modality as CT. For future work we wish to continue our in vivo studies with the 30.6±5.0 nm diameter polyMAOETIB nanoparticles. For this purpose, conditions to prepare stable concentrated saline dispersions of the iodinated nanoparticles, e.g. 10–15%, would be developed. In parallel, we will explore their toxicity, biocompatibility and pharmacokinetics behavior.

III. X-RAY PHOTOELECTRON SPECTROSCOPY :

1. INTRODUCTION :

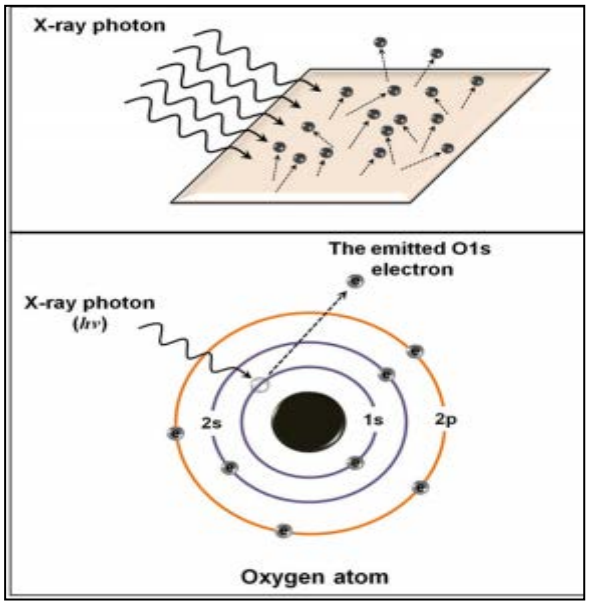
The field of biomaterials is inherently multidisciplinary involving materials science, physical, engineering, biological and clinical sciences. Therefore, it is important for biomaterials researchers to understand what to and how to characterize different biomaterials, so the study of surfaces and interfaces using X-rays has undergone considerable developments in recent years. One of most usable methods that used x-ray in biomaterials is XPS (x-ray photoelectron spectroscopy).

2. DEFINITION:

X-ray photoelectron spectroscopy (XPS), which is also called electron spectroscopy for chemical analysis, is the most widely used analytical technique to monitor the surface chemistry of solid materials due to its simplicity, flexibility, and sound theoretical basis. The typical XPS instrument includes an ultra-high vacuum system, X-ray source, electron energy analyzer, and data acquisition system. In XPS, the sample surface is irradiated by monochromatic X-ray and the emitted photoelectrons are detected. Figure 1 describes the general mechanism of photoelectron creation and the energy of the photoelectron is given by:

$E_B = hv\text{-}KE;$

where EB is the binding energy of the electron in the atom, hv is the energy of the X-ray source (a known value in the experiment), and KE is the kinetic energy of the emitted electron that is measured. EB is usually expressed in electron volts (eV) and 1 eV = 1.6 10^{19}J. Since there are different electrons and binding energies in an atom, each element produces a set of unique peaks in the photoelectron spectrum and the peak intensity is a direct measure of the elemental concentration. As XPS is extremely surface sensitive, surface contamination can lead to stray results. XPS can provide qualitative and quantitative information on all elements except hydrogen and helium and furthermore, the shape of each peak and the exact binding energy can be slightly altered by the chemical state of the emitting atom. Hence, XPS can provide chemical bonding information as well. XPS is typically performed by first taking a survey scan covering a range of 1000 eV and then, smaller energy ranges indicative of specific features are subsequently scanned in higher resolution. Survey scans are often in low resolution and are used to identify as well as quantify in terms of atomic percentage of major elements present on the materials surface. The x-axis is generally the ‘binding energy’ and the y-axis is typically ‘intensity’ or ‘number of counts’.



Figure(Top) A surface irradiated by X-ray photons resulting in emission of photoelectrons and (bottom) the X-ray photon transfers its energy to a core-level electron imparting enough energy for the electron to leave the atom.

3. PRINCIPE:

3.1 INSTRUMENTATION:

-Electron energy analyzer: measures the KE of emitted e^-s.

- X-ray source

- Neutralizer

-Vacuum system $<10^{-9}$Torr ($<10^{-7}$Pa): will prevent contamination of the surface and aid an accurate analysis of the sample.

- Electronic controls

- Computer system: The computer system will control the X-Ray type and prepare the instrument for analysis.

-Detection of electrons

-Avoid surface reaction/contaminations

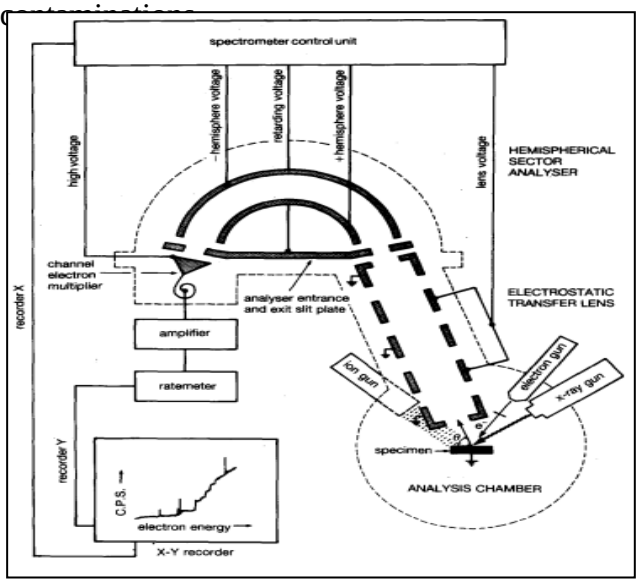


Figure:Instrumentation of XPS

- The sample is irradiated with monochromatic X-rays Cause the ionization of its atoms by photoelectric effect. The kinetic energy Ec of these photoelectrons is measured, which gives the Spectrum of the intensity of the electrons as a function of the measured energy. Each incident X-ray has the same energy $h \cdot \nu$, since the beam is Monochromatic (h being the Planck constant and ν the frequency of the wave Incident light).
- During the interaction with the atom, part of this energy is used to break Binding is binding energy, EL; the remainder is transferred to the electron under the Form of kinetic energy.

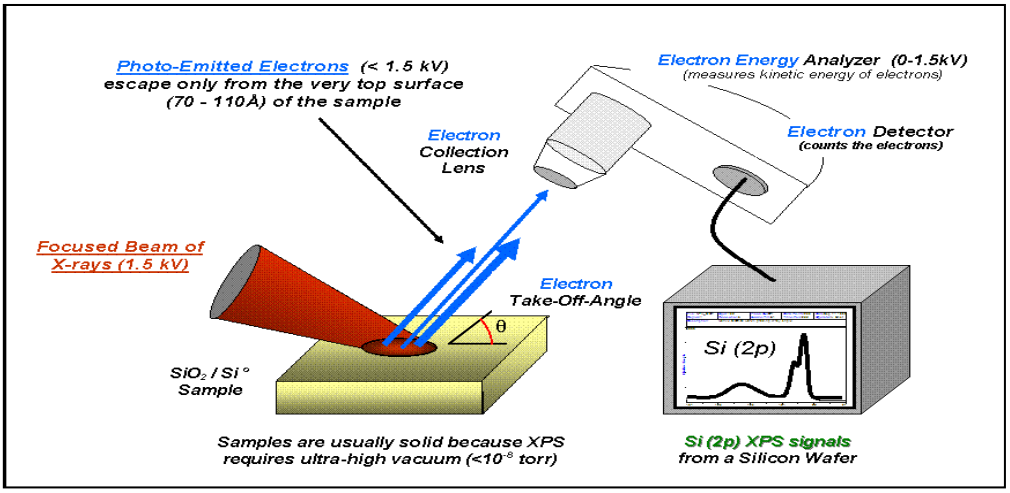


Figure explaining of XPS method

3.1.MEASUREMENT:

XPS is used to measure:

- elemental composition of the surface (top 0 –10 nm usually)
- empirical formula of pure materials
- Elementsthatcontaminate a surface
- chemical or electronic state of each element in the surface
- uniformity of elemental composition across the top surface (or line profiling or mapping)
- uniformity of elemental composition as a function of ion beam etching (or depth profiling)

Example

To understand how to identify and quantify elements from the survey XPS data. As an example, the typical survey spectra acquired from biomedical polytetrafluoroethylene films before and after plasma surface modification are illustrated in Fig.2

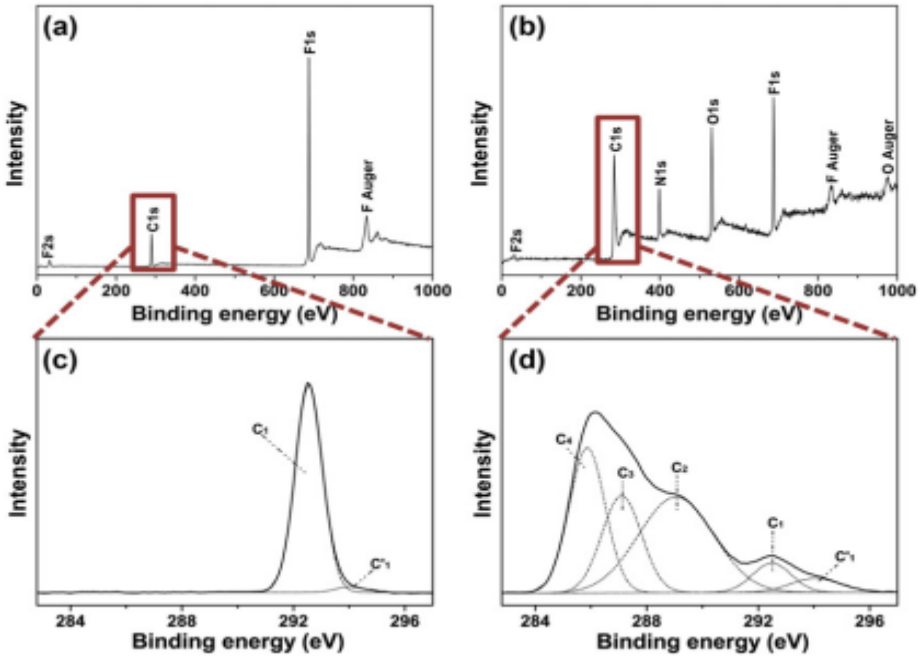


FIGURE 2 (a, b) XPS survey spectra and (c, d) high-resolution C1s spectra (c, d) acquired from biomedical polytetrafluoroethylene (PTFE) films before (a, c) and after (b, d) nitrogen/ ammonia plasma immersion ion implantation: C1 (F–C*–F), C01 (C1 at the end of polymer chains),C2 (–C*¼O, –C*¼NH, –C*–F), C3 (–C*–NH2, –C*–OH), and C4 (–C*–H, –C*–C–).

The energy of the incident photon X is of the order of magnitude of the ionization energy of the core electrons: their emission gives the XPS peaks essentially characteristic of the nature of the atom; whereas the chemical information (especially the degree of oxidation) is derived from the small displacements of the XPS peak corresponding to the energy variation between valence layers, the latter (corresponding to the UV / visible / near IR domains in general) is small compared to that of X-rays.

XPS – some characteristics:

- XPS is a quantitative technique (like AES).
- Extracts information from top 1 to 12 nm of material.
- Uses ultra high vacuum.
- XPS detects elements with $Z \geq 3$ (lithium); cannot detect H or He as these atoms are too small.
- Detects in the parts per thousand limit; parts per million is possible from top surfaces and long collection time.
- XPS can be used to analyze inorganic compounds, metal alloys,

semiconductors, polymers, glasses, ceramics, paints, paper, inks, wood, plant parts, bones, bio-materials, oils, glues, surface contamination, etc

• XPS uses narrow beams of 20 - 200 micrometers of monochromatic Al Kα X-rays or broad 10 – 30 mm beam of non-monochromatic Mg X-rays.

4. Like any methods this one has advantages and disadvantages:

Advantages:

- Non-destructives
- Surface-sensitive
- Quantitative measurement
- Provides information about chemicalbonding

Disadvantages:

- Veryexpansive
- high vacuum isrequired
- Slow processing
- H and He can’t be detected.

CONCLUSION:

The field of biomaterials is inherently multidisciplinary involving materials science, physical, engineering, biological and clinical sciences. Therefore, it is important for biomaterials researchers to understand what to and how to characterize different biomaterials. So the application of appropriate surface analysis techniques based on X-ray for correlating surface properties, including chemical structure, hydrophobic it y, morphology and topography, and material performance is of paramount importance , it's our main goal to use X-ray in other fields like biomaterials because of their important relation with biocompatibility.

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