INTERNATIONAL COLLEGE OF PHARMACEUTICAL INNOVATION
国际创新药学院

Chemical and Microbiological stability in Solution Dosage Forms

Course BSc(Pharm) & BSc (ATT)

Year 2024-2025 II

Module Medicines Pharmaceutics 2

Lecturer Dr. Congcong Xu

LEARNING OUTCOMES

- An understanding of the different types of solutions
- Describe the different sources of microbial contamination
- Discuss the role of regulations in the control of microbial contamination in sterile and non sterile products
- Discuss the effect of microbial contamination on sterile products
- Describe the role of antimicrobial agents
- Describe the main methods of chemical instability
- An understanding of the methods by which these instabilities can be avoided

RECOMMENDED READING

Part 3: Pharmaceutical microbiology and sterilization

PHARMCEUTICAL SOLUTIONS

- Non – sterile
  - Solutions
  - Suspensions
  - Elixirs酏剂

- Sterile
  - Injections/vaccines
  - IV fluids
  - Eyedrops
  - Irrigation fluids灌洗液

NON STERILE SOLUTIONS

- Easy to swallow
- Therapeutic response often faster
- Homogeneous system
- Taste masking of bitter therapeutic agents easy to achieve
- Irritation is reduced because of immediate dilution

- *Suitable media for growth of microorganisms*
- *Stability often poorer than tablets or capsules*
- Shelf life is often shorter
- Bulky to transport
- Often unpleasant taste

STERILE SOLUTIONS

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the human or animal body

Examples

- Injections
- Vaccine
- IV fluids
- Eye drops
- Irrigation fluids

Need to be free from bacterial or pyrogen, physical or chemical contamination

Formulation: active, vehicle, preservative, antioxidant, chelating agent, buffer etc

AUTOCLAVE (STEAM STERILIZATION)

EFFECT OF STERILIZATION

Table 15.1 Death of *Bacillus megaterium* spores in pH 7.0 buffer at 95 °C

Time (min)	Viable cell concentration (mL^{-1})	Percentage of survivors	Log_{10} percentage of survivors
0	2.50×10^6	100	2.000
5	5.20×10^5	20.8	1.318
10	1.23×10^5	4.92	0.692
15	1.95×10^4	0.78	−0.108
20	4.60×10^3	0.18	−0.745
25	1.21×10^3	0.048	−1.319
30	1.68×10^2	0.0067	−2.174

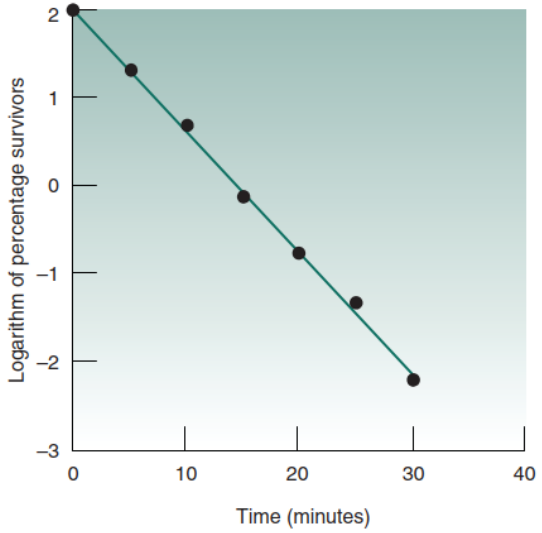


Fig. 15.1 Heat inactivation of *Bacillus megaterium* spores at 95 °C.

Table 16.1 Time and temperature combinations used for moist and dry heat sterilization

Process	Minimum temperature (°C)	Minimum holding period (min)
Steam sterilization (autoclaving)	115	30
	121	15
	126	10
	134	3
Dry heat	140	180
	150	150
	160	120
	170	60
	180	30

Solutions are a suitable media for growth of microorganisms

NON – STERILE SOLUTIONS

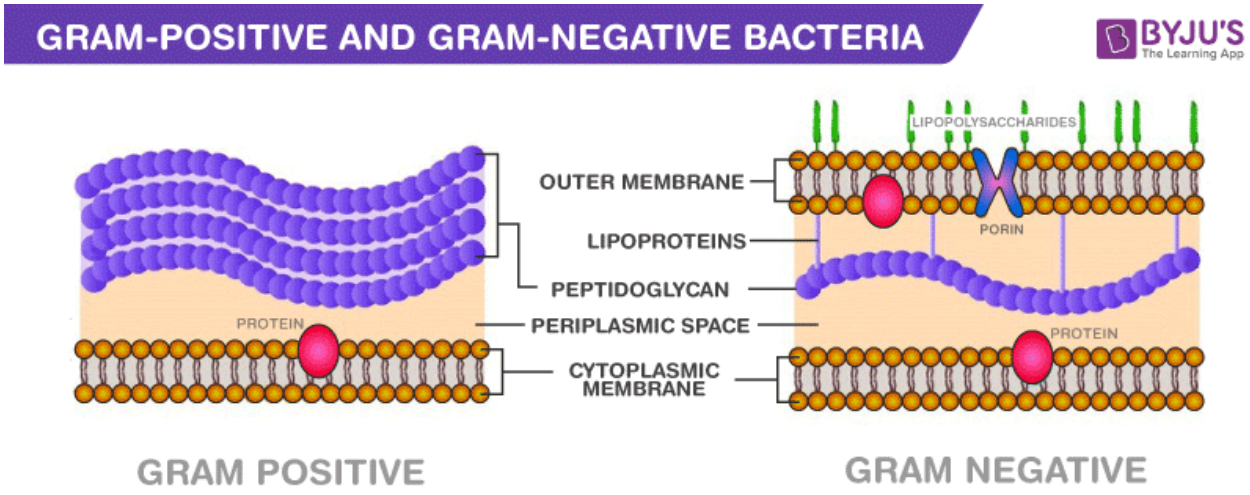
- Oral solutions are non-sterile
- There are restrictions on the number and type of microorganism present:
  - European Pharmacopoeia states that in oral products
    - E Coli must be absent
    - There should not be more than 1000 aerobic bacteria（好氧细菌）
    - Not more than 100 fungi（真菌）per gram or mL
- Multi-dose
  - Necessary to inhibit growth of bacteria and fungi
- Populations using these oral dosage forms includes new-borns, paediatrics and geriatrics who may not be able to take oral solid dosage forms and may be compromised, defective dosage forms can pose a greater risk because of the population being dosed

- Microbes presented in drugs not only makes them hazardous from the infectious standpoint, in addition may change the chemical, physical and organoleptic properties of the drugs or change the contents of active ingredients.

SOURCES OF MICROBIAL CONTAMINATION

- Microorganisms are ubiquitous in nature
- They can adapt and survive under a variety of conditions
- All sources of entry should be considered when making a product
- CGMP – current good manufacturing practice

GRAM-POSITIVE VS GRAM-NEGATIVE



	Gram-positive bacteria	Gram-negative bacteria
Color after gram testing	Blue or purple	Pink or red
Cell wall thickness	Thick	Thin
Toxins	• emetic toxin • diarrheal enterotoxins • neurotoxins • enterotoxin	endotoxins

ENDOTOXIN VS EXOTOXIN

(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.

(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

Exotoxins are usually heat labile proteins secreted by certain species of bacteria which diffuse into the surrounding medium.

Endotoxins are heat stable lipopolysaccharide-protein complexes which form structural components of cell wall of Gram Negative Bacteria and liberated only on cell lysis or death of bacteria.

STERILE VS PYROGEN-FREE

FEATURES	STERILE	PYROGEN-FREE
Definition	The term Sterile means the absence of viable microorganisms [1] [2].	The term Pyrogen-free means the absence of Pyrogen.
Testing Method	A product is sterile that is confirmed by the sterility test. According to U.S.P sterility test is done by two basic methods: 1. Direct inoculating the test sample on a suitable culture medium at optimum conditions for bacterial growth 2. Filtration Technique.	A product is pyrogen-free that is confirmed by the pyrogen test. According to B.P. two types of test are available for pyrogen test: 1. Rabbit Test 2. LAL (Limulus Amoebocyte Lysate)鲎hòu试剂Test
Method to do	Sterilization is a process to make a product sterile. Sterilization is done by the following method [2]: 1. Physical Method a. Dry heat sterilization b. Moist heat sterilization c. Sterilization by radiations 2. Chemical method a. Gaseous sterilization b. Sterilization by disinfectants 3. Mechanical methods a. Sterilization by filter	Depyrogenation is a process to make a product pyrogen-free. Depyrogenation is done by the following method: 1. Ultrafiltration 2. Reverse osmosis 3. Affinity chromatography 4. Dilution or rinsing 5. Distillation 6. Adsorption 7. Hydrophobic attachment 8. Acid or base hydrolysis 9. Ionizing radiation 10. Moist heat 11. Dry heat
Example	Example of Some Sterile preparations are parenteral, ophthalmic, irrigating preparations and medical devices	Example of Some Pyrogen-free preparations are Water for injection, SVP (Small Volume Parenteral) LVP (Large Volume Parenteral) and medical devices

LIMULUS AMOEBOCYTE LYSATE TEST

EU PHARMACEUTICAL INFORMATIONS

All topics

Go back to **EU Pharmaceutical informations** > Eudralex

EU Legislation - Eudralex

The body of European Union legislation in the pharmaceutical sector is compiled in Volume 1 and Volume 5 of the publication "The rules governing medicinal products in the European Union".

- Volume 1 - EU pharmaceutical legislation for medicinal products for human use
- Volume 5 - EU pharmaceutical legislation for medicinal products for veterinary use

The basic legislation is supported by a series of guidelines that are also published in the following volumes of "The rules governing medicinal products in the European Union":

- Volume 2 - Notice to applicants and regulatory guidelines for medicinal products for human use
- Volume 3 - Scientific guidelines for medicinal products for human use
- Volume 4 - Guidelines for good manufacturing practices for medicinal products for human and veterinary use
- Volume 6 - Notice to applicants and regulatory guidelines for medicinal products for veterinary use
- Volume 7 - Scientific guidelines for medicinal products for veterinary use
- Volume 8 - Maximum residue limits
- Volume 9 - Guidelines for pharmacovigilance for medicinal products for human and veterinary use
- Volume 10 - Guidelines for clinical trial

Medicinal products for paediatric use, orphan, herbal medicinal products and advanced therapies are governed by specific rules

EudraLex - Volume 4 Good manufacturing practice (GMP) Guidelines.

Volume 4 of "The rules governing medicinal products in the European Union" contains guidance for the interpretation of the principles and guidelines of good manufacturing practices for medicinal products for human and veterinary use laid down in Commission Directives 91/356/EEC, as amended by Directive 2003/94/EC, and 91/412/EEC respectively.

Introduction

- Introduction (33 KB) (7/02/2011)
- Commission Directive 2003/94/EC, of 8 October 2003, laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use
  Replacement of Commission Directive 91/356/EC of 13 June 1991 to cover good manufacturing practice of investigational medicinal products.
- Commission Directive 91/412/EEC of 23 July 1991 laying down the principles and guidelines of good manufacturing practice for veterinary medicinal products.

Part I - Basic Requirements for Medicinal Products

- Chapter 1 Pharmaceutical Quality System (65 KB) (into operation since 31 January 2013)
- Chapter 2 Personnel (58 KB)(into operation since 16 February 2014).

CONTROL OF MICROBIAL CONTAMINATION IN NON STERILE PRODUCTS

- The USP Microbiological Attributes Chapter <1111> provides little specific guidance other than

  "The significance of microorganisms in non-sterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user."
- The USP recommends that certain categories be routinely tested for total counts and specified indicator microbial contaminants.
- For example natural plant, animal and some mineral products for Salmonella, oral liquids for E. Coli, topicals for P. aeruginosa and S. Aureus, and articles intended for rectal, urethral, or vaginal administration for yeasts and molds.
- A number of specific monographs also include definitive microbial limits.

WHAT ARE STERILE SOLUTIONS USED FOR?

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STERILE PRODUCTS MANUFACTURE

Sterile products must be

- Freedom from pathogens
- Freedom from pyrogens
- Freedom from particulates

Achieved through

- Terminal sterilisation
- Aseptic manufacture无菌生产

WHY??

Delivery bypasses protective chemistry of the GIT and the low permeability barrier of the skin

Filled into single use ampoules安瓿bù瓶 or multidose (requires addition of preservative)

ANTIMICROBIAL AGENTS

- Function : inhibit growth of microrganisms
- Added to products which are packaged in multiple dose vials
- May be added to single dose parenterals which cannot be terminally sterilised
  - Trend to eliminate use of antimicrobials
- Not used in large volume injections- BP single dose volume >15ml
- Not if drug/drug formulation has sufficient antimicrobial activity

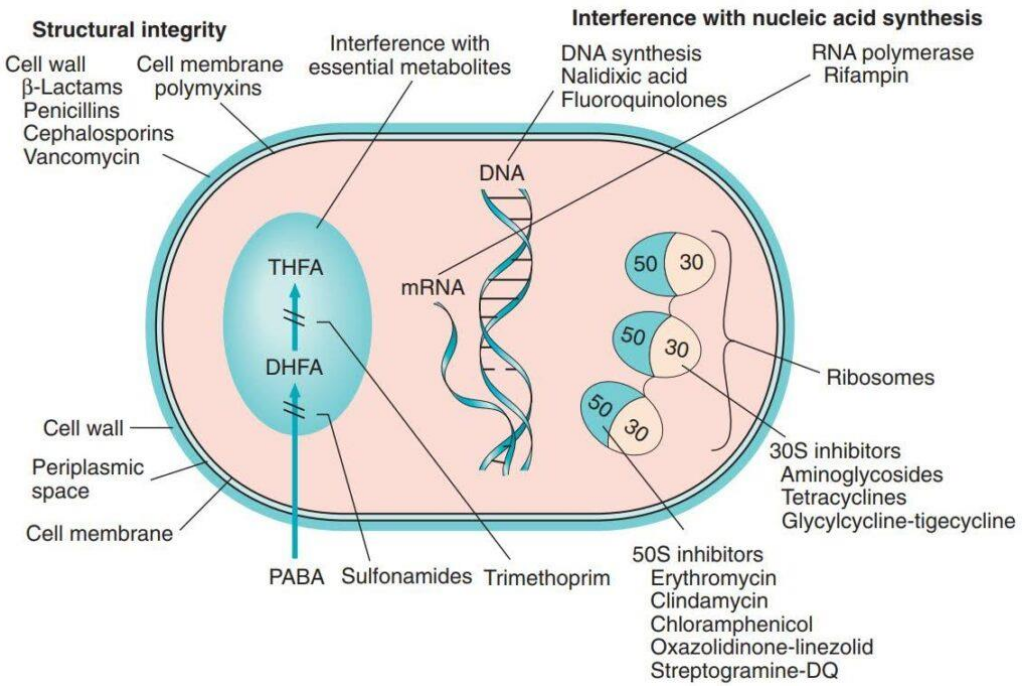
Examples

- Benzalkonium Chloride苯扎氯铵
- Benzyl alcohol苯甲醇
- Chlorobutanol氯丁醇
- Phenol苯酚
- Chlorocresol氯甲酚
- Phenylmercuric salts苯汞盐
- Methylhydroxybenzoate对羟基苯甲酸甲酯

MECHANISM OF ACTION OF ANTIMICROBIAL AGENTS

There are around 23 distinct classes and 18 subclasses of clinically antibacterial drugs, totaling approximately 100 antibiotics. The classification of these antibiotics is complex and continuously evolving. Their mechanisms of action include targeting bacterial cell wall synthesis, folate synthesis, DNA replication, RNA transcription, and mRNA translation. These metabolic processes are sufficiently distinct from those in eukaryotic cells, allowing for selective toxicity. Understanding these mechanisms also provides insights into how microorganisms develop resistance.

1.Inhibition of cell wall synthesis
2.Inhibition of cell membrane function
3.Inhibition of protein synthesis
4.Inhibition of nucleic acid synthesis



MECHANISM OF ACTION OF ANTIMICROBIAL AGENTS

STERILE SOLUTIONS REGULATIONS

- Legally binding documents
  - EU GMP annex 1
    - Contains guidance to minimise risk of contamination by
      - Microbes
      - Particles
      - Pyrogens
  - European Pharmacopoeia
    - Contains monographs on the manufacture and testing of formulations
  - PIC/S
    - The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

EU GMP ANNEX 1 - STERILE MEDICINAL PRODUCTS

EU GMP Annex 1- Basic Elements

- Clean room classification
- Monitoring
- Technologies
- Personnel
- Premises
- Equipment
- Sanitation
- Processing
- Sterilisation Methods
- Aseptical Filling
- Finishing

EUROPEAN COMMISSION
ENTERPRISE AND INDUSTRY DIRECTORATE-GENERAL

Consumer goods
Pharmaceuticals

Brussels, 25 November 2008 (rev.)

EudraLex
The Rules Governing Medicinal Products in the European Union

Volume 4
EU Guidelines to
Good Manufacturing Practice
Medicinal Products for Human and Veterinary Use

Annex 1
Manufacture of Sterile Medicinal Products
(corrected version)

Pseudobacteremia caused by povidone-iodine **solution contaminated** with Pseudomonas cepacia

DE Craven, B Moody, MG Connolly... - ... England Journal of ..., 1981 - Mass Medical Soc

... Contamination of hospital disinfectants with Pseudomonas species. **...** Frank MJ, Schaffner W. **Contaminated** aqueous benzalkoniUm chlo- ride: an unnecessary hospital infection hazard. JAMA. **...** False positive blood cultures: association with non- **sterile** blood collection tubes. **...**

Cited by 138 Related articles All 3 versions Web of Science: 93 Cite Save More

An outbreak of Candida parapsilosis bloodstream infections in patients receiving parenteral nutrition

SL Solomon, RF Khabbaz, RH Parker... - Journal of Infectious ..., 1984 - jid.oxfordjournals.org

... pump was used in the pharmacy docu- mented reflux of **contaminated** pump contents into **sterile solutions**. **...** The presence of HSA in fluids that **contaminated** the pump may have enhanced the growth of yeasts to an extent that permitted **contamination** of compounded **...**

Cited by 137 Related articles All 5 versions Web of Science: 88 Cite Save More

Nosocomial Pseudomonas pickettii colonization associated with a **contaminated** respiratory therapy **solution** in a special care nursery.

MM McNeil, SL Solomon, RL Anderson... - Journal of clinical ..., 1985 - Am Soc Microbiol

... DISCUSSION This is the second reported incident of intrinsic contami- nation of a **sterile solution** with P. pickettii. The first epi- demic associated with intrinsic **contamination** occurred in Great Britain (7). The epidemic involved **contaminated** wa- ter, purified in a hospital **...**

Cited by 50 Related articles All 7 versions Web of Science: 35 Cite Save More

Contaminated enteral nutrition **solutions** as a cause of nosocomial bloodstream infection: a study using plasmid fingerprinting

J Levy, Y Van Laethem, G Verhaegen... - Journal of Parenteral ..., 1989 - pen.sagepub.com

... A 1-ml aliquot is obtained with a **sterile** syringe and rapidly shipped on ice to **...** before administration to the patient demon- strated that when a **solution** was **contaminated**, low bacterial **...** performed by the infection control nurse to determine the source of **contamination** revealed the **...**

Cited by 87 Related articles All 6 versions Web of Science: 49 Cite Save More

Multistate Nosocomial Outbreak of Ralstonia pickettii Colonization Associated with an Intrinsically **Contaminated** Respiratory Care **Solution**

JA Labarca, WE Trick, CL Peterson... - Clinical infectious ..., 1999 - cid.oxfordjournals.org

... R. pickettii has a propensity to **contaminate** different **solutions**, as demonstrated by **...** In addition, several pseudo-outbreaks due to laboratory **contamination** have been reported [11–13 **...** We identified intrinsically **contaminated** "**sterile**" 0.9% sodium chloride **solution** used for tracheal **...**

Cited by 56 Related articles All 8 versions Web of Science: 43 Cite Save More

Aseptic peritonitis due to peptidoglycan **contamination** of pharmacopoeia standard dialysis

EFFECT OF MICROBIAL CONTAMINATION OF STERILE PRODUCTS

- Case study 1:
- 2012 New England Compounding Centre
- caused death of 64 patients and over 800 sick
- compounded epidural steroid硬膜外麻醉注射类固醇 injections containing methylprednisolone甲基强的松龙
- CDC found fungal matter in contaminated methylprednisolone
- Fungus was found in the cerebrospinal fluid脑脊液of several patients and caused meningitis脑膜炎
- Between May and September 2012 patients in 23 states received injections from three lots of contaminated medication

EFFECT OF MICROBIAL CONTAMINATION OF STERILE PRODUCTS

- Case study 2:
  - Nine deaths occurred among 19 patients who developed Serratia marcescens bacteremia粘质沙雷氏菌血症in six Alabama hospitals that were infusing contaminated total parenteral nutrition [TPN] solutions produced by a compounding pharmacy
  - TPN solutions resulted in identification of multiple breaches involving compounding and sterilizing amino acids for TPN formulations
  - Enterobacteria Gram negative沙门氏菌 uniformly resistant to a wide range of antibiotics including narrow-spectrum-penicillins 青霉素 and cephalosporins头孢菌素, cefuroxime头孢呋辛, cephamycins头霉素, macrolides大环内酯类, tetracycline四环素, nitrofurantoin呋喃妥英and colistin粘菌素
  - Causes pneumonia, lower respiratory tract infection, urinary tract infection, bloodstream infection, wound infection and meningitis脑膜炎

CHEMICAL STABILITY

Chemical stability is concerned with maintaining the integrity of the chemical structure of the active ingredient

Small drug molecules prone to:

- Hydrolysis
- Oxidation
- Photolysis光分解
- Racemization外消旋作用

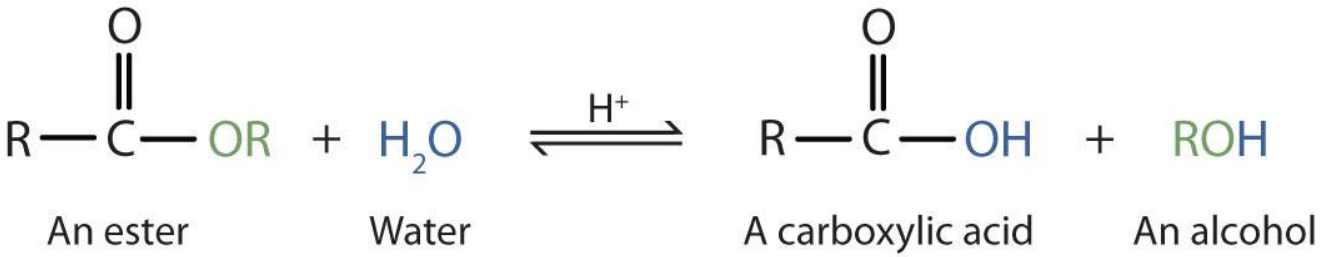
Proteins and nucleic acids are prone to denaturation, aggregation and adsorption.

HYDROLYSIS

Splitting of drugs with water

Most susceptible groups are carboxylic acid derivatives that spilt into carboxylic acid and other groups

Oxygen of water attacks the carbonyl carbon (electron deficient) yielding carboxylic acid and in the case of the ester the alcohol



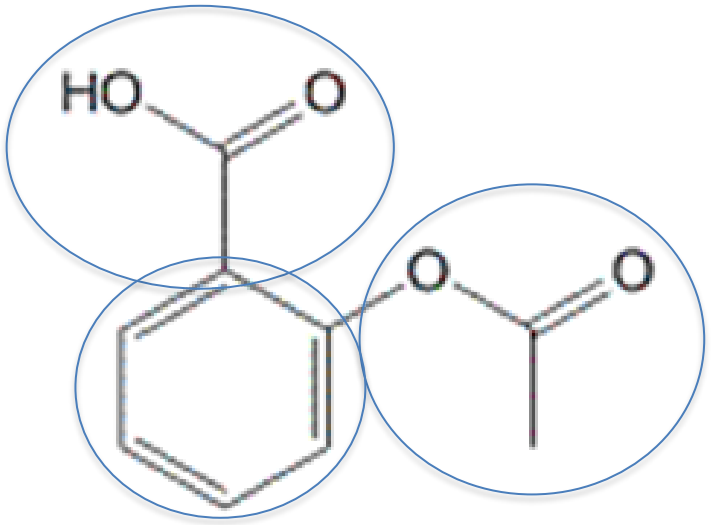
HYDROLYSIS

- Most important in systems containing water such as emulsions, suspensions, solution etc
- Also for drugs affected by moisture from atmosphere (packaging considerations)
- Main classes of drugs that undergo hydrolysis are Esters, Amides酰胺, Alkalis碱and Acids

CASE DRUG: ASPIRIN HYDROLYSIS

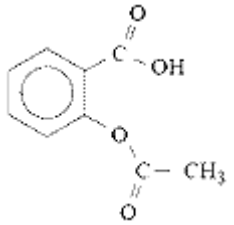
Aspirin (acetylsalicylic acid) contains three groups:

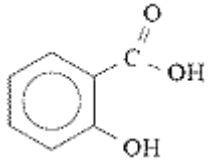
1. carboxylic acid functional group (R-COOH)
2. ester functional group (R-O-CO-R') – methyl ester most prone to hydrolysis
3. aromatic group (benzene ring)



acetylsalicylic acid

CASE DRUG: ASPIRIN HYDROLYSIS





aspirin (acetylsalicylic acid)	+	water	→	salicylic acid	+	acetic acid (ethanoic acid)
$C_9H_8O_{4(s)}$	+	$H_2O_{(l)}$	→	$C_7H_6O_{3(s)}$	+	$C_2H_4O_{2(aq)}$

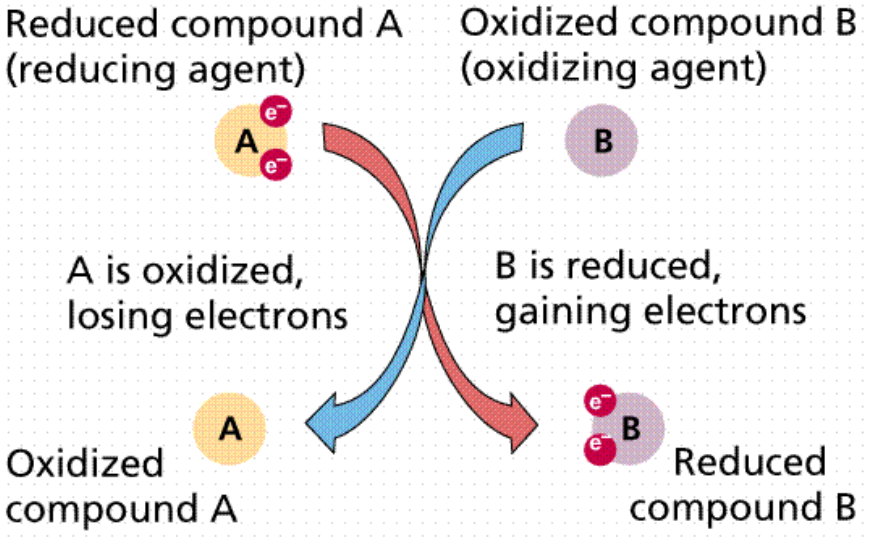
Old aspirin tablets may have a smell like _____ as a result of the hydrolysis reaction producing acetic acid (ethanoic acid乙酸).

AVOIDING HYDROLYSIS

- Avoid contact with moisture during manufacture
- Packaging in strip packs and storage in controlled humidity and temp
- In liquid dose form optimum pH should be calculated and solution buffered accordingly
- Formulate as tablets or dry powder rather than liquids

http://www.medicines.ie/ingredient/79/Aspirin/

OXIDATION



- Involve the loss of electrons
- Opposite of reduction (gain of electrons)
- Mostly catalysed by enzymes
- Often catalysed by colour changes in the drug solution
- Chain reaction
- Involves formation of a free radical (drug with unpaired electron designated $R^{\cdot}$)
- Initiation – formation of free radical
- Propagation – free radical takes an electron from another drug molecule producing another free radical
- Termination – two free radicals find each other – electrons are paired and reaction stops

OXIDATION AND PHARMACEUTICALS

- Oxidation is usually mediated through reaction with atmospheric oxygen under ambient conditions (autoxidation自然氧化)
- Sensitivity to oxidation can be worked out by investigating stability in an atmosphere of high oxygen
- Oxidation reactions can be catalysed by oxygen, heavy metal ions and light which lead to the formation of free radicals
- Aldehydes, alcohols, phenols酚类, alkaloids生物碱类, unsaturated fats and oils are all susceptible to oxidation

AVOIDING OXIDATION

- Replace air in containers headspace with nitrogen
- Control pH
- Add in chelating agents which stabilise drugs by trapping metals responsible for oxidation initiation
  - Fe^{3+}, Cu^{2+}
- Add in antioxidants which are preferentially oxidised compounds (O_2 scavengers)
  - Sodium metabisulphite焦亚硫酸氢钠
  - Sodium bisulphate亚硫酸氢钠
  - Ascorbic acid维生素C
  - Tocopherols维生素E
- Keep at low temps where they are more stable because of reduced activity of metal ions

CONTAMINATION AND STABILITY

Important to know:

- Active drug
  - Oxidation potential
  - Structure
  - Stability
- Other excipients in formulation

- Delivery method
  - Sterile/not sterile
  - Sterile – single or multi dose?
  - Route of administration

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