CHAPTER II
LITERATURE REVIEW

2.1 Type of Wounds

A wound is defined as an injury or tear on the skin surface by physical, chemical mechanical and thermal damages. There are two types of wounds.

2.1.1 Acute Wounds

The acute wounds are caused by traumas, but the wounds are usually healable within 8 to 12 weeks. These wounds can be caused by mechanical damage induced by sheer, blunting, or hard action. The acute wounds can also be formed by exposure to extreme heat, irradiation, electrical shock, and/ irritated with corrosive chemicals. The Caring for these wounds depend on the severity of the wounds (Zahedi *et al.*, 2009).

2.1.2 Chronic Wounds

Chronic wound are open wound extending into at least the second layer of the skin called the dermis. The injuries which are produced as a result of specific diseases such as diabetes, tumors, and severe physiological contaminations. Healing of these wounds could take more than 12 weeks and recurrence of the wounds is not uncommon (Zahedi *et al.*, 2009).

2.2 Principles of Wound Healing

Wound healing is a special biological process which is related to physiological parameters. These can be summarized into five phase of healing process includinghemostasis, inflammation, migration, proliferation, and maturation (Zahedi *et al.*, 2009). These phases of wound healing are shown in **Fig.1 (a–d)**, respectively.

2.2.1 Inflammatory Phase

This stage occurs after there is damage to the skin, your body's normal response to injury. This phase activates protective measures through chemical activity causing heat, redness, pain, swelling, loss of function. Fibrinogen, which is one of the major components of the skin's connective tissues leads to the coagulation of exudates (blood without cells and platelets), and together with the formation of a fibrin network, produces a clot in the wound which stops.

2.2.2 Migratory Phase

The new and live cells called epithelial move towards skin injury to replace dead cells.

2.2.3 Proliferative Phase

This is the time when your wound is healing. Your body makes new blood vessels, which cover the surface of the wound. The result is that your wound will become smaller as it heals. The completion of this stage takes about 2 weeks.

2.2.4 Maturation Phase

This is the final phase of healing. At this stage fibroblasts completely cover the surface of the wound as a new layer of the skin and there is no evidence of the wound.

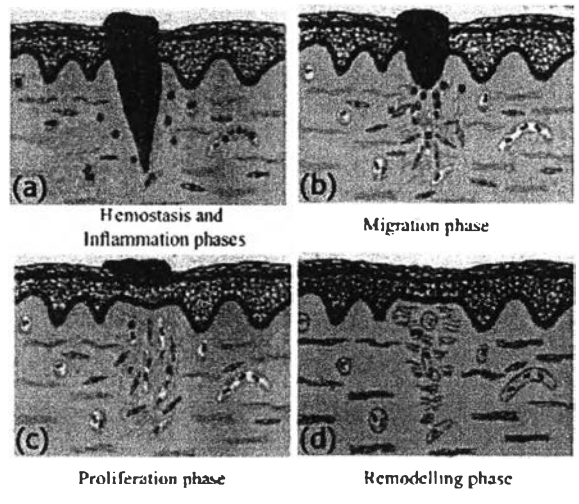


Figure 2.1 Schematic representation of different phases of wound healing (Zahedi *et al.*, 2009).

2.3 Wound Dressings and Properties

Wound dressing should minimize infection and pain, prevent excessive fluid loss, maintain a moist healing environment, promote epithelial restoration, and biocompatible (Thakur *et al.*, 2008). Applications of dressing material are aimed to inhibition of bleeding and protection of the wound from environmental irritants including water and electrolyte disturbances (Jayakumar *et al.*, 2011).

2.3.1 Classification of Wound Dressings

2.3.1.1 Ordinary Dressings

The Ordinary dressings that is gauze and tulle, merely act as a common cover on a wound so that the wound can rehabilitate underneath.

2.3.1.2 Interactive Dressing

The interactive dressing that is materials containing polymeric films, and/or foams which are transparent and permeable to water vapor and atmospheric oxygen such as hyaluronic acid, hydrogels and foamed covers.

2.3.1.3 Bioactive Dressing

The Bioactive dressing that is materials or in other words active wound dressing materials such as hydrocolloids, alginates, collagens and chitosan.

2.3.2 Properties of Wound Dressing (Watson *et al.*, 2005)

- Maintains a moist environment around the wound
- Removes excess exudate, but prevents saturation of the dressing.
- Permits diffusion of gases
- Protects wound from micro-organisms
- Provides mechanical protection
- Controls local temperature and pH
- Easy and comfortable to remove or change
- Minimizes pain from the wound
- Does not contaminate the wound
- Low cost

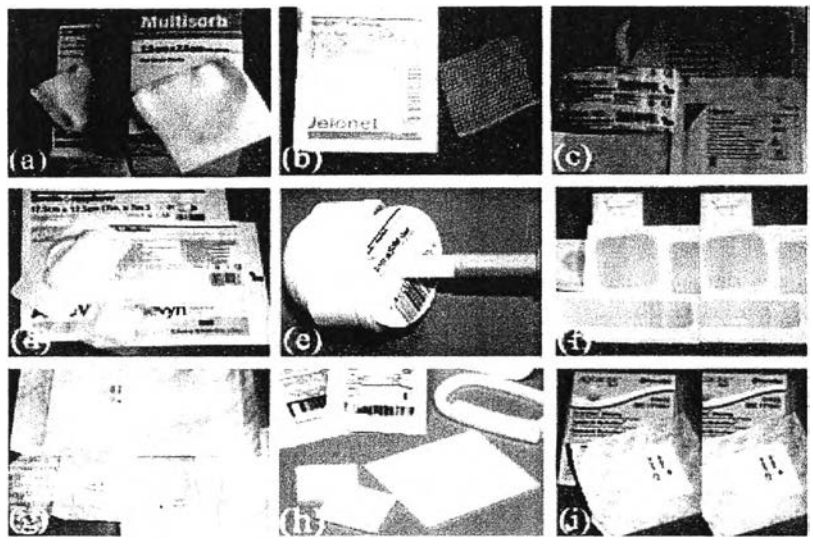


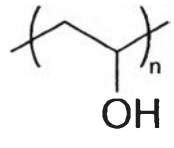
Figure 2.2 Show different types and appearances of passive, interactive, and bioactive wound dressing materials: (a) gauze, (b) tulle, (c) polyurethane membrane, (d) polyurethane foam, (e) hydrogel, (f) hydrocolloid, (g) alginate, (h) collagen, and (i) hydrofiber. (Zahedi *et al.*, 2009)

2.4 Poly(Vinyl Alcohol) or PVA

Poly(vinyl alcohol) is a hydrophilic polymer, biocompatibility and odorless synthetic polymer. PVA is granular and white, and only dissolves in hot water. It is usually prepared by dissolving it in water, and letting the water evaporate to form transparent films with exceptional strength and resistance to tearing (Tupureina *et al.*, 2009).

2.4.1 Chemical Structure

The structure of poly (vinyl alcohol) is given below:



2.4.2 Manufacturing

PVA is not prepared by polymerization of the corresponding monomer. The monomer, vinyl alcohol, is unstable with respect to acetaldehyde.

PVA instead is prepared by first polymerizing vinyl acetate, and the resulting polyvinyl acetate is converted to the PVA. The conversion of the polyesters is usually conducted by base-catalyzed transesterification with ethanol. The properties of the polymer depend on the amount of residual ester groups.

2.4.3 Properties

2.4.3.1 Physical and Chemical Properties

PVA's flash point is 79 degrees Celsius, while its melting point is less than or equal to 200 degrees Celsius. Its density ranges from 1.19 to 1.31 g/cm^3. It can also release toxic fumes when burned, and reacts with strong acids and oxidants. It is essentially nontoxic for human beings as long as it is not burned or melted with fire. There is also no proof that PVA posts any inhalation risk and physical dangers to humans.

2.4.3.2 *Adhesive, Emulsifying and Film-forming Properties*

PVA has excellent adhesive, emulsifying and film-forming properties. Because of its excellent adhesive property A PVA sponge is also available, and this particular sponge can absorb water up to 12 times its dry weight. It can also withstand 90 degrees Celsius without experiencing any deformation. As a film, PVA becomes transparent and resistant to tearing and punctures.

2.4.3.3 Resistance to Impurities

PVA is inherently resistant to organic solvents, oil and grease. It is used as a grease-proof coating and unit packages for medical equipment and tools to ensure they are free from any impurities. It is also an impermeable material to most natural gases; ensuring materials packed in PVA will not be contaminated.

2.4.4 PVA for Wound Dressing Applications

Previous researcher studied and prepared wound dressing by using PVA act as material in vary from including film, nanofiber and hydrogel wound dressing.

(Tupureina *et al.*, 2009) and coworker prepared wound dressing by combining PVA with bentonite, cellulose, Ag nanoparticles, rosemary extract and clove extract, via the freezing/thawing method. In addition, the wound dressing

present high water absorption capacity, gelcontent, and a water vapor transmission rate similar to the natural skin and excellent antimicrobial activity indicating its ability to act as an effective wound dressing material.

(Hang *et al.*, 2010) and coworker prepared mats of a poly(vinyl alcohol) (PVA)/chitosan (CS) blend (PVA/CS) and PVA/CS blends incorporated with silver (Ag) nanoparticles (Ag/PVA/CS) were fabricated by an electrospinning method. The material showed good antibacterial activity against the gram-negative bacteria *E.coli.* The antibacterial activity of non-woven mats of Ag/PVA/CS blends was better than that of non-woven mats of PVA/CS blends.

(Vicentini *et al.*, 2010) and coworker prepared chitosan (CS) and poly(vinyl alcohol) (PVA) film blend with ZnO nanoparticles, were prepared by the Pechini method, with different concentrations of polyoxyethylene sorbitan monooleate, Tween 80. In these films, antibacterial activity was verified toward the species *S.aureus*, attributed to the incorporation of ZnO nanoparticles into the films.

(Huang *et al.*, 2008) and coworker prepared aqueous mixture of β-glucan and poly(vinyl alcohol) by casting into films and dried at 110 °C without chemical crosslinking. This PVA/glucan films exhibited negligible irritation to skin. On observing the wound healing of rat skin, the wound contraction ratio can reach 83% after treating with PVA/glucan film for 11 days, while that was 85% when treating with cotton gauze for 21 days.

2.5 Chitosan

2.5.1 Structure of Chitosan

Chitosan is a natural polysaccharide consisting of β-(1-4)-2-acetamido-2-deoxy-d-glucose(N-acetyl D-glucosamine) and β-(1-4)-2-amino-2-deoxy-d-glucos (D glucosamine) as repeating units (Rinaudo, 2006). Chitosan presents in **Fig 2.3** which is derived from the partial deacetylation of chitin in **Fig 2.4**, which is obtained from the shells of crustaceans, cuticles of insect, and cell wall of fungi and yeasts. The deacetylation degree (DD) of chitosan, giving indication of the

number of amino groups along the chains, is calculated as the ratio of D-glucosamine to the sum of D-glucosamine and N-acetyl D-glucosamine

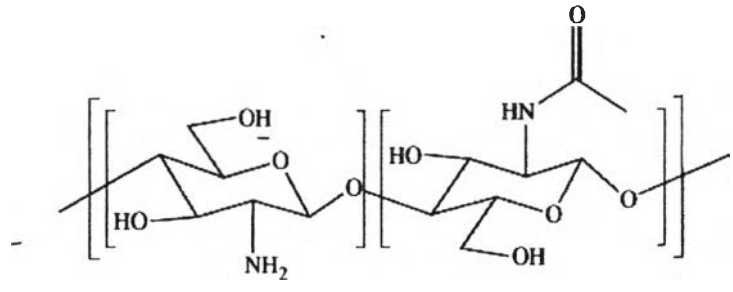


Figure 2.3 Chemical structure of chitosan

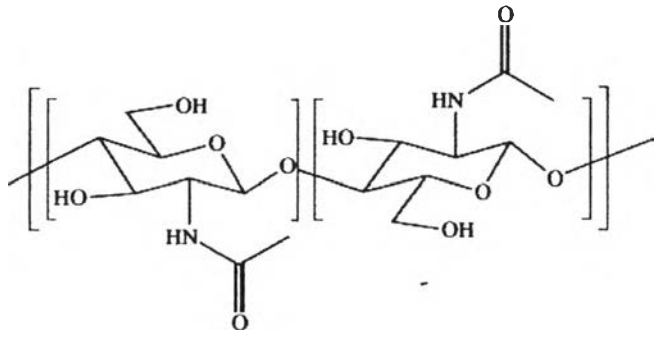


Figure 2.4 Chemical structure of chitin

2.5.2 Physicochemical Properties of Chitosan

The presence of amino groups in the chitosan structure and gives to this polymer many peculiar properties. Indeed, the amino groups of the D-glucosamine residues might be protonated providing solubility in diluted acidic aqueous solutions (pH < 6). The relative molecular weight of chitosan range from hundreds of thousands to millions. Chitosan and its derivatives have widely used in pharmaceutical and medical compatibility, biodegradability, antibacterial activity.

Chitosan is a polycation whose charge density depends on the degree of acetylation and pH. So, chitosan chains are able to interact by electrostatic

interactions with negatively charged molecules. It can form nanoparticles by ionic gelation with polyphosphates (Calvo *et al.*, 1997) and with nucleic acids.

Chitosan offers remarkable biological properties, which have paved the way for its application in the pharmaceutical and biomedical fields (He *et al.*, 1998) in new drug delivery systems (Pardakhty *et al.*, 2007) or as a scaffold for tissue engineering. Indeed, chitosan has good adhesive properties due to its positive charge (Lehr *et al.*, 1992) which increases the adhesion to mucosa and so the time of contact for drug penetration. Their hemostatic property makes chitosan a good candidate for wound dressing (Minagawa *et al.*, 2007).

2.5.3 Chemically Modified Chitosan

The amino functionality of chitosan gives rise to chemical reactions such as acetylation, quaternization, reactions with aldehydes and ketones (to give Schiff's base) alkylation, grafting, chelation of metals, etc.(Pillai *et al.*, 2009). The N-Trimethyl, chitosanchloride (TMC), a quaternized CS derivative, has been proven to effectively increase the permeation of hydrophilic molecule for improving water solubility (Sayin *et al.*, 2009) (Yang *et al.*, 2005) and coworker have prepared the chitosan derivatives through the reductive N-alkylation of chitosan as described by (Badawy *et al.*, 2011) with various mono- and disaccharides. Methylated N-(3-pyridylmethyl) chitosan chloride (M3-PyMeChC) with various degrees of N-substitution (DS), degrees of quaternization (DQ), and molecular weights were synthesized by single methylation of N-(3-pyridylmethyl) chitosan with iodomethane under the basic condition which reported by (Sajomsang *et al.*, 2009). A series of N-(2-hydroxy)propyl-3-trimethyl ammonium chitosan chloride (HTCC) samples with various degrees of quaternization ranging from 12.4 to 43.7% was synthesized by (Xiao *et al.*, 2012). N-pyridylmethyl chitosan and its quaternized derivatives were synthesized in order to enhance the solubility, the physicochemical and biological properties (Sajomsang, 2010).

2.6 Nanoparticle in Drug Delivery

In recent years, significant efforts have been devoted to use the potentials of nanotechnology in drug delivery since it offers a suitable means of site-specific and

time-controlled delivery of small or large molecular weight drugs and other bioactive agents (Yih *et al.*, 2006). Pharmaceutical nanotechnology focuses on formulating therapeutically active agents in biocompatible Nano forms such as nanoparticles, nanocapsules, micellar systems, and conjugates. These systems offer many advantages in drug delivery, mainly focusing on improved safety and efficacy of the drugs, e.g. providing targeted delivery of drugs, improving bioavailability and improving the stability of therapeutic agents against chemical/enzymatic degradation. The Nano scale size of these delivery systems is the basis for all these advantages. By a general definition, nanoparticles vary in size from 10 to 1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a boundary structure.

Several types of nanoparticulate systems have been attempted as potential drug delivery systems, including biodegradable polymeric nanoparticles, polymeric micelles, solid nanoparticles, lipid-based nanoparticles, e.g., Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC), nanoliposomes, inorganic nanoparticles, dendrimers, magnetic nanoparticles, Ferro fluids and quantum dots (Hamidi *et al.*, 2008).

2.6.1 Chitosan Nanoparticles with Ionic Crosslinks

The cationic nature of chitosan has been conveniently exploited for the development of particulate drug delivery systems. Aside from its complexation with negatively charged polymers. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic chitosan by electrostatic forces for drug encapsulation: **Fig 2.5** this simple and straightforward technique involves the addition of an alkaline phase (pH ~ 7–9) containing TPP into an acidic phase (pH ~ 4–6) containing chitosan. Nanoparticles are formed immediately upon mixing of the two phases through inter and intra molecular linkages created between TPP phosphates and chitosan amino groups (Agnihotri *et al.*, 2004)

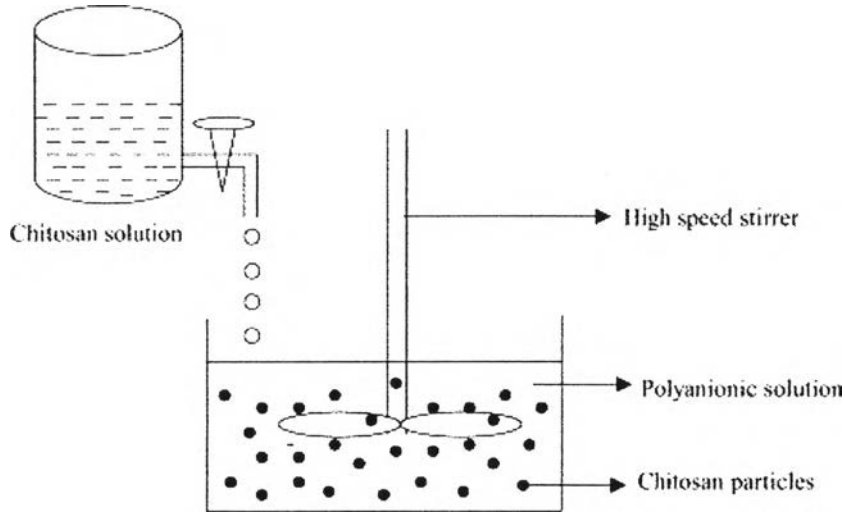
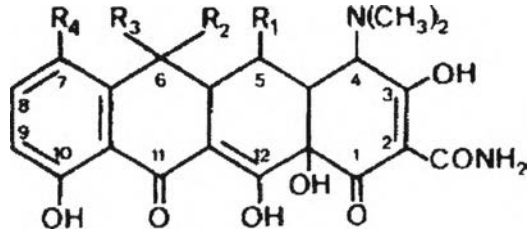


Figure 2.5 Schematic representation of preparation of chitosan particulate systems by ionic gelation method (Agnihotri *et al.*, 2004).

2.7 Tetracycline

The tetracycline, which was discovered in the 1940s, is a broad-spectrum antibiotic produced by the Streptomyces genus of action bacteria, indicated for use against many bacterial infections. It is a protein synthesis inhibitor. It is commonly used to treat acne today, and, more recently, rosacea, and is historically important in reducing the number of deaths from cholera. Tetracycline is marketed under the brand names Sumycin, Tetracyn, and Panmycin, among others. It is also used to produce several semisynthetic derivatives, which together are known as the tetracycline antibiotics. Tetracycline has in common a four-ring carboxylic structure. Differences between them are substitutions at position 5, 6 and 7 in **fig 2.6** (Chopra, 2002).



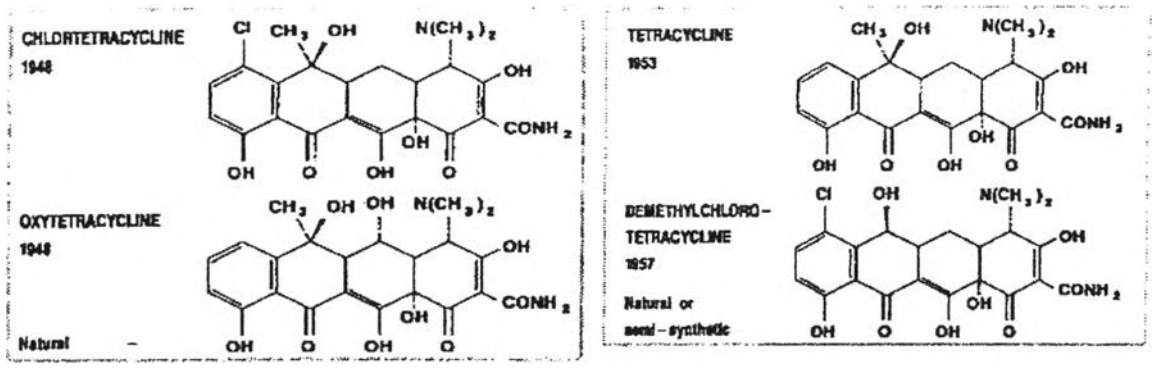


Figure 2.6 fundamental structures of tetracycline derivatives showing the most important positions (5, 6, 7) for substitutions.

2.7.1 Mode of Action

It is well established that tetracycline inhibit bacterial protein synthesis by preventing the association of aminoacyl tRNA with the bacterial ribosome. Therefore, to interact with their targets these molecules need to traverse one or more membrane systems depending on whether the susceptible organism is gram positive or gram negative (Chopra *et al.*, 2001). Access to the ribosomes of gram-negative bacteria is obtained by passive diffusion through hydrophilic pores in the outer cell membrane and then by an energy-dependent active transport system that pumps all tetracycline through the inner cytoplasmic membrane.

2.7.2 Resistance to Tetracycline (Gialdroni Grassi, 1993)

Resistance can be expressed through different biochemical mechanisms. Classically, it is assumed-that while in susceptible bacterial cells there is an accumulation of tetracycline; in resistant ones an energy-dependent efflux prevents accumulation of the drug in the cell. This mechanism of pumping out the drug is mediated by a resistance protein inserted into the bacterial cytoplasmic membrane. So far, this is the main mechanism of tetracycline resistance in *Aeromonas, Haemophilus, Pasteurella* and *Pseudomonas* spp. Two new mechanisms of resistance have recently been discovered in other groups of bacteria **(Fig 2.7)**: namely, ribosomal protection whereby a cell component, presumably a protein, binds to the ribosome rendering it less susceptible to tetracycline; and chemical modification of the tetracycline molecule determined by bacteria through an

oxygen-requiring reaction, and rendering the drug inactive. The exact mechanisms of these two types of resistance have not yet been fully elucidated. In the case of ribosomal protection, two hypotheses have been put forward on the basis of some experimental findings. Resistance could be due to reduced binding of tetracycline to the ribosome, or to weak binding to ribosomal sites other than the primary ones, probably because the distortion exerted by the resistant protein on the ribosome makes the secondary site more accessible. However, it has not been assessed whether the protein encoded by the resistance gene acts directly on ribosomes or modifies a host protein, to which the protective activity is devolved.

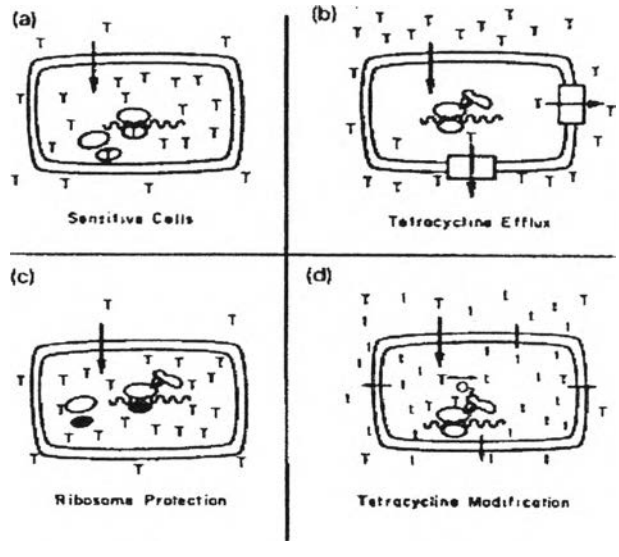


Figure 2.7 schematic representations of the mode of action of tetracycline in sensitive cells and the possible mechanisms of resistance (a) Tetracycline-penetrates into sensitive bacterial cell, reaching much higher concentrations than the extracellular environment; it binds to the 30S ribosomal subunit inhibiting protein synthesis. (b) The classical mechanism of resistance; tetracycline cannot reach a sufficient intracellular concentration due to an increased efflux. (c) Ribosomal protection mechanism of resistance; tetracycline concentrate intracellular, but ribosome is modified in such a way that the drug cannot bind effectively to it. (d) Tetracycline modification type of resistance; tetracycline concentrate intracellular but are rendered inactive by an oxygen-requiring chemical reaction (Salyers *et al.*, 1990)