Development of new generation wound care products is an evolving field today and to find clinical and commercial niche for emerging wound management products, one needs to have proper understanding of process involved in wound healing and their treatment in more advanced way.

1.1 Wounds
The skin is the largest organ of the body and has many different functions. The epidermis or outer layer is made of mostly dead cells associated with protein called keratin [Tortora GJ; 1993]. This makes the layer waterproof and is responsible for protection against the environment [Ralph HG; 1982]. The dermis or middle layer is made up of living cells. It also has blood vessels and nerves that run through it and is primarily responsible for structure and support. The subcutaneous fat layer is primarily responsible for insulation and shock absorbency [meddean.luc.edu].

Wound refers to a sharp injury, which damages the dermis of the skin. Wound can be described as separation of tissues of the body, an injury due to external violence, or an imperfection. The repair of an epithelial wound is merely a scaling up of this normal process. Science of wound healing is recorded as "three healing gestures" on a clay tablet, one of the oldest medical texts dated 2200 BC. It describes the three gestures as; washing the wound; Making plasters; and Bandaging the wound [woundcarestrategies.com]. Although there has been a significant advancement in today's science of wound healing the basic theme seems to be similar [Rayn TJ; 1993]. The work of Joseph Lister and Louis Pasteur established a sound basis for the management of infection by identifying the cause and developing methods for preventing wound. Louis Pasteur proved that bacteria did not spontaneously generate but were introduced into wounds from a foreign source. These findings encouraged Lister's advocacy of frequent washing with soap and water and fueled his search for ways to kill bacteria, or the "antiseptic technique" a major advance in the field of wound healing. The antiseptic technique was followed shortly by the "aseptic technique," in which a sterile environment was used to prevent the onset of infection [woundcarestrategies.com].

Wounds are generally classified as, wounds without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments e.g. venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and dermal abrasions. Wounds are also classified by the layers involved, superficial wounds involve only the
epidermis, and partial thickness wounds involve epidermis and dermis, while full thickness wounds also involve the subcutaneous fat or deeper tissue. Although restoration of tissue continuity after injury is a natural phenomenon, infection, quality of healing, speed of healing, fluid loss and other complications that enhance the healing time represent a major clinical challenge. Majority of wounds heal without any complication. However, chronic non-healing wounds involving progressively more tissue loss give rise to the biggest challenge to wound-care product researchers. Unlike surgical incisions where there is very little tissue loss and are easy to heal, chronic wound disrupt normal process of healing and is often not sufficient in itself to affect repair. Delayed healing is a result of compromised wound physiology [Cohen IK; 1998] and occurs with venous stasis, diabetes, or prolonged local pressure. Second major challenge is the prevention of scarring, keloid formation or contractures and a cosmetically acceptable healing.

A wound can have a significant impact on a person’s life. Wounds can lead to prolonged periods of disability in addition to suffering pain and discomfort, and may even prevent a person from performing everyday activities such as walking and bathing. This inactivity may in itself lead to further health problems. Some wounds are associated with odour and excessive drainage and require frequent attention as they may impede social interactions. A non-healing wound may prevent a return to work which can have psychological as well as economic ramifications [Figure 1.1].

\[Figure 1.1\] Impact of wound on quality of life
1.1.1 Wound healing

Wounding destroys various layers of skin and the underlying soft tissues. Following injury, wound healing follows a series of tightly regulated, sequential events. These are inflammation, granulation tissue formation, reepithelization, and remodeling (Figure 1.2). Haemostatics and hydrogels have a multifaceted role in the mediation of these cellular and matrix events [Kerstein MD; 1997].

The phases of wound healing are:

- **Hemostasis**
- **Inflammation**
- **Proliferation or Granulation**
- **Remodeling or Maturation**

Kane’s analogy to the repair of a damaged house provides a wonderful framework to explore the basic physiology of wound repair [Kane D; 2001] (See Table 1.1).

Table 1.1 Phases of wound healing

<table>
<thead>
<tr>
<th>Phase of healing</th>
<th>Days post injury</th>
<th>Cells involved</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemostasis</td>
<td>Immediate</td>
<td>Platelets</td>
<td>Haemostatics</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Day 1-4</td>
<td>Neutrophils</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>Proliferation</td>
<td>Day 4-21</td>
<td>Macrophages</td>
<td>Hydrogel/ film</td>
</tr>
<tr>
<td>Granulation</td>
<td>Day 4-21</td>
<td>Lymphocytes</td>
<td>Hydrocolloid</td>
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<tr>
<td></td>
<td></td>
<td>Angiocytes</td>
<td>Skin equivalents</td>
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<td></td>
<td></td>
<td>Neurocytes</td>
<td>Skin grafts</td>
</tr>
<tr>
<td>Contracture</td>
<td>Day 21- 2 yrs</td>
<td>Fibroblasts</td>
<td>Gel sheets</td>
</tr>
<tr>
<td>Remodelling</td>
<td></td>
<td>Fibrinocytes</td>
<td>Collagenase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Keratinocytes</td>
<td>Radiation therapy</td>
</tr>
</tbody>
</table>
**Hemostasis:**
In wound healing the *platelet* is the cell which acts for sealing off the damaged blood vessels. The blood vessels themselves constrict in response to injury but this spasm ultimately relaxes. The platelets secrete vasoconstrictive substances to aid in this process but their prime role is to form a stable clot sealing the damaged vessel. Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues the platelets aggregate and adhere to the exposed collagen [MacLeod J; 1981]. They also secrete factors which interact with and stimulate the intrinsic clotting cascade through the production of *thrombin*, which in turn initiates the formation of fibrin from fibrinogen. The fibrin mesh strengthens the platelet aggregate into a stable haemostatic plug. Finally platelets also secrete cytokines such as platelet-derived growth factor (PDGF), which is recognized as one of the first factors secreted in initiating subsequent steps. Hemostasis occurs within minutes of the initial injury unless there are underlying clotting disorders.

**Inflammation Phase:**
Clinically inflammation, the second stage of wound healing presents as erythema, swelling and warmth often associated with pain, the classic “rubor et tumor cum calore et dolore”. This stage usually lasts up to 4 days post injury. The inflammatory response causes the blood vessels to become leaky releasing plasma and Poly Morpho Nucleocytes into the surrounding tissue. The neutrophils phagocytize debris and microorganisms and provide the first line of defense against infection. They are aided by local *mast cells*. As fibrin is broken down as parts of this clean-up the degradation products attract the next cell involved [Wahl LM, et.al; 1992]. Macrophages plays major role in this phase. They phagocytize bacteria and provide a second line of defense. They also secrete a variety of chemotactic and growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming
growth factor beta (TGF and interleukin-1 (IL-1) which appears to direct the next stage [Kerstein MD; 1994].

**Proliferative Phase (Proliferation, Granulation and Contraction):**
The granulation stage starts approximately four days after wounding and usually lasts until day 21 in acute wounds depending on the size of the wound. It is characterized clinically by the presence of pebbled red tissue in the wound base and involves replacement of dermal tissues and sometimes subdermal tissues in deeper wounds as well as contraction of the wound.

Fibroblasts secrete the collagen framework on which further dermal regeneration occurs. Specialized fibroblasts are responsible for wound contraction. Pericytes regenerate the outer layers of capillaries and the endothelial cells which produce the lining. This process is called as angiogenesis. The keratinocytes are responsible for epithelialization. In the final stage of epithelialization, contracture occurs as the keratinocytes differentiate to form the protective outer layer or stratum corneum [Wahl LM, et.al; 1992].

**Remodeling or Maturation Phase:**
Wound repair or the healing process involves remodeling the dermal tissues to produce greater tensile strength. The principle cell involved in this process is the fibroblast. Remodeling can take up to 2 years after wounding.

In healthy individuals with no underlying factors an acute wound should heal within three weeks with remodeling occurring over the next year or so. If a wound does not follow the normal trajectory, it may get stuck in one of the stages and the wound becomes chronic. Chronic wounds are defined as wounds, which have “failed to proceed through an orderly and timely process to produce anatomic and functional integrity, or proceeded through the repair process without establishing a sustained anatomic and functional result” [Lazarus G, et.al; 1994].

1.1.2 Wound Management

Wound management is careful and accurate assessment of the wound with the use of proper wound care products. Over the years the market has moved from traditional (gauze based) products to advanced (moist wound healing) products to actives (antimicrobials, mechanical devices). There are a handful of organized companies in the international market dealing with wound management products, like 3M, Johnson & Johnson, Elder-Hartmann and Smith & Nephew. Some international brands used for
wound management include Coloplast, Convatec, Lohmann & Rauscher and Molnlycke [Turner TD; 1979].

In a stark contrast to developed markets, the smallest segment in the Indian wound care market for advanced wound care consists of high-technology products like hydrocolloids, hydrogels, alginates, foams, film dressings and other scar or burn management products.

Until 1960, advances in the design and efficacy of wound management products have been spasmodic and limited to the adaptation of available materials that were being used for other purposes. The products were primarily of the plug and conceal variety, and could be considered passive products that took no part in the healing process. Very little attention was paid to the functional performance of a product and minimal consideration was given to the healing environments required for different wound types [Morgan DA; 1999].

A new generation of products was potentiated by the advances in knowledge. The humoral and cellular factors associated with the healing process and the realization that a controlled microenvironment was needed if wound healing was to progress at the optimum level [Prescribing nurse bulletin; 1999].
The ultimate goal of wound management is the prevention of wounds, or the halting of wound deterioration to achieve more rapid healing. This goal can only be accomplished by intervening with appropriate quality care, in a timely manner with appropriate wound management.

There are three basic principles which underlie wound healing:

1. Identify and control as best as possible the underlying causes
2. Support patient centered concerns
3. Optimize local wound care

**Factors to be considered while optimizing wound management**

**Decreased dehydration and cell death:** As described earlier, the task of wound repair requires the activity of a host of cells from neutrophils and macrophages to fibroblasts and pericytes. These cells cannot function in a dry environment [Winter GD; 1962].

**Increased angiogenesis:** Not only do the cells required for angiogenesis need a moist environment but also angiogenesis occurs towards regions of low oxygen tension such that occlusive dressings may act as a stimulus in the process [Knighton DR et. al; 1981].

**Enhanced autolytic debridement:** By maintaining a moist environment neutrophil cell life is enhanced and proteolytic enzymes are carried to the wound bed allowing for painless debridement [Baxter CR; 1994]. Further these fibrin degradation products also stimulate macrophages to release growth factors into the wound bed.

**Increased re-epithelialization:** In larger, deeper wounds epidermal cells must spread over the wound surface from the edges. They must have a supply of blood and nutrients. Dry crusted wounds reduce this supply and provide a barrier to migration thus slowing rates of epithelialization [Haimowitz JE, et.al; 1997].

**Bacterial barrier and decreased infection rate:** Occlusive dressings with good edge seals can provide a barrier to migration of microorganisms into the wound. Bacteria have been shown to pass through 64 layers of moist gauze [Mertz PM, et.al; 1985]. Wounds covered with occlusive dressings have reported to have lower rates of infection than those with conventional gauze dressings [Hutchinson JJ, et.al; 1980].

**Decreased pain:** It is believed that the moist wound bed insulates and protects the nerve endings thereby reducing pain. Furthermore occlusive dressings require fewer dressing changes, which may be comfortable for patients.

**Decreased costs:** While occlusive dressings have a higher per unit cost than conventional gauze, the reduced frequency of dressing changes and increased healing rates may prove
Introduction

Design and development of surgical dressings for advanced wound management to be cost effective in the long term. While moist wound healing has clear advantages, debate continues on how moist is moist. Dressings should retain enough moisture to stimulate good healing and yet should not cause maceration or irritation to the surrounding tissues [Rich A; 1998].

1.1.3 Ideal wound dressing

It is widely accepted that a warm, moist wound environment encourages healing and prevents tissue dehydration and cell death. These conditions also allow the interaction of the cells and growth factors involved in the in healing process (Figure 1.2) [Morgan DA; 1999]. Therefore, ideal wound dressing should:

- maintain a moist environment at the wound surface
- Provide thermal insulation.

In addition, the dressing should:

- provide mechanical protection and protect against secondary infection
- be non-adherent and easily removed without trauma
- leave no foreign particles in the wound
- remove excess exudate without allowing ‘strike through’ to the surface of the dressing
- be cost effective and offer effective pain relief.

Over the past few years an ever-expanding list of dressing products has come onto the market in an attempt to meet these conditions. Among them are the transparent film dressings, hydrogels, hydrophilic foams, alginates, hydrocolloids and the newer
antibacterial and biological dressings or devices. There is however no magic “one-size-fits-all” dressing. The clinician needs to become familiar with the characteristics of the different classes of dressings and to tailor the dressing used to the phase of healing, characteristics of the wound, the needs (and risk factors) of the patient, the availability and skills of the caregiver.

Based on available evidence and clinical experience, modern dressings appear to have a role at various stages of the wound healing process. Though no single product is suitable for all wound types or at all stages of healing, Absorbable haemostats and hydrogel dressings are the part of modern wound management systems which help in haemostasis and moist wound healing respectively. Haemostasis initiates the wound healing and hydrogels enhance proliferation of the wound.

1.1.4 Advanced Wound Management

Tremendous strides have been made in wound management since the mid-1980s when transparent films and hydrocolloids began to replace traditional gauze pads and non-adherent dressings as primary coverings for acute and chronic wounds. Today, Advanced Wound Management dressings including hydrocolloids, alginates, gels and foams allow healthcare professionals to manage moisture at the wound surface and reduce the frequency of dressing changes from several times a day to several times a week [Haimowitz JE, et.al; 1997].

New wound care technologies are being developed at an increasingly rapid pace in recent years. These innovations could significantly reduce overall costs for treating complex and chronic wounds, while offering greater savings in preventing wounds and their recurrence.

Advanced wound management includes products for managing difficult-to-heal wounds. These include chronic wounds such as pressure and diabetic foot ulcers as well as burns and post operative wounds. The management of chronic wounds is a challenging task for the healthcare system. Aggressive management of the underlying conditions causing chronic wounds as well as patient individualized goal setting is central to cost effective wound care. Once these objectives are achieved, topical management of the wound with appropriate dressing to support healing and avoidance of infection are vital adjuants [dressing.org].
Dressings such as hydrogels, hydrocolloids and films may efficiently maintain moisture or manage an excess amount of moisture due to exudates in a more resource effective manner than moist gauze dressings. These products are designed to help speeding up the healing process and improving patient’s quality of life. Advanced polymeric gels, films and foams that create a moist environment, encourage healing, and antimicrobial dressings and ointments can be used for treating and preventing wound infection [woundcarestrategies.com].

Potential benefits of advanced wound management products to patients include fewer reapplications of dressings, less discomfort and pain, faster healing and reduced risk of complications such as infection and amputation. Treatment with such products would be clinically effective and less time-consuming and therefore more cost effective [Rayn TJ; 1993].

1.1.5 Wound dressings
Wound dressings are an essential component of every wound care treatment plan. Wound dressings may vary from simple to complex

Classification of wound dressing [dressing.org]:

a. Based on its nature of action

![Figure 1.3 Classification of dressing based on its nature of action](image)

Passive dressings simply provide cover, while active dressings serve to actively change the environment of a wound. Biologic dressings add materials which may be depleted. Traditional dressings like gauze and tulle dressings that account for the largest market segment are passive products. Active dressings comprise of polymeric films and forms, which are mostly transparent, permeable to water vapour and oxygen but impermeable to
bacteria. These films are recommended for low exuding wounds. Biologic dressings are constructed from materials having endogenous activity. These materials include proteoglycans, collagen, non-collagenous proteins, alginates or chitosan. In November 1999, Food and Drug Administration of the United States of America (USFDA) reclassified the dressing’s categories as,
1. Non-resorbable gauze/sponge dressing for external use,
2. Hydrophilic wound dressings,
3. Occlusive wound dressings,
4. Hydrogel wound and burn dressings and
5. Interactive wound and burn dressings

b. Based on usage

![Classification of dressings based on nature of action](image)

Figure 1.4 Classification of dressings based on nature of action

c. Based on type of materials used

![Classification of dressings based on type of materials used](image)

Figure 1.5 Classification of dressings based on type of materials used
**Synthetic Dressings**

Synthetic polymers derived from petroleum products can be easily manufactured using conventional technology into films, fibers, sheets, and sponges. For this reason these materials have received attention as potential wound dressings for deep wounds [www.smtl.uk].

Synthetic polymers have several advantages such as ability to adhere to the wound edges, ability to drape to the wound contour and ease of use. The major disadvantage is the lack of biological properties such as enhancing wound healing via attraction of cells involved in healing process [Taylor MM, et.al; 2001].

These dressings are used as coverings for deep (full-thickness) burns and skin ulcers. In these applications synthetic polymeric dressings create an inert environment that controls water and heat passage from the wound while preventing bacterial infiltration.

Examples of some synthetic polymeric wound dressings are:

- **Conventional dressings:** Gauze, Lint, Gauze swab, Nonwoven viscose swab.
- **Paraffin gauze dressings:** Open mesh nylon fabric, Nonwoven pad coated with aluminum.
- **Hydrocolloids:** Natural hydrocolloid and polyurethane foam layers.
- **Hydrogels:** Polyacrylamide, Agar and Sodium carboxymethylcellulose and polyurethane films.
- **Others:** Biomembranes: Silicone rubber membrane; knitted nylon fabric and collagen peptides.

**Biological Dressings**

Biological dressings are derived from natural tissues usually consisting of various formulations and combinations of collagen, elastin and lipids. They are far superior to synthetic dressings in that they

1. Restore water vapor barrier and prevent dehydration of the wound;
2. Decrease evaporational heat loss;
3. Decrease protein and electrolyte losses in wound exudates;
4. Prevent bacterial contamination of the wound and hence protect the wound and patient from sepsis;
5. Permit less painful dressing changes;
6. Permit painless movement over joints;
7. Facilitate debridement of wounds;  
8. Create good granulation tissue bed for autografting of deep wounds;  
9. Can be used to test for successful subsequent autograft;  
10. Decrease healing time of partial thickness burns and donor sites and  
11. Improve quality of healing, inhibit excessive fibroblasts and decrease contraction 

**Biodegradable dressings**  

**a) Collagenous Dressings**  
Porous collagenous materials employ 3-D porous structure (sponge) in the design of a dermal dressing. A three-dimensional structure allows tissue ingrowth into the material and with time the wound tissue and implant cannot be separated. The implant is ultimately degraded and is replaced by normal scar tissue [Babu M, et.al; 2000, David B; 2008].  

**b) Alginate Dressings**  
They are composed of sodium alginate extracted from brown seaweeds. Sodium alginate is water soluble and can be converted to insoluble calcium salt, which is then formed into films. The calcium salt is manufactured as mats for wound dressings, ropes or balls for deeper wounds, and ribbons for packing sinuses. Other dressings include a percentage of sodium alginate to increase the rate of gel formation and useful in treating dry or lightly exuding wounds. The dressings are useful in the management of burns and donor sites, leg ulcers, and infected traumatic wounds [Thomas S; 2000]  

**c) Film Dressings**  
They are homogeneous dressings composed of a polymer sheet coated on one side with an adhesive. They are highly elastomeric and transparent. The most commonly used polymers include polyurethane, polyethylene, polycaprolactone, polytetrafluoroethylene, dimethyl amino ethyl methacrylate. Film dressings are well suited for superficial wounds, but owing to lack of absorbency and impermeability to water vapour and gases, they may cause accumulation of wound fluid beneath the dressing and hence allow leakage of exudates and entry of exogenous bacteria to the wound surface. Therefore, they are not convenient for larger wounds [dressing.org].  

**Examples:** Polyurethane, Copolymer of dimethylaminoethyl methacrylate and acetonitrile, Hydroxyethylmethacrylate (HEMA) and silastic film, Poly(ε-caprolactone)
film, Poly(ethoxyethylmethacrylate)film, Silicone polymer membrane, Poly (hydroxyethylmethacrylate) (HEMA) and polyethylene glycol, Copoly(D,L-lactide).

d) Foam Dressings
The main properties of foams are their conformability, pore size, insulation and increased adherence to wounds. Polymers such as polyurethane are used to make foams that are stabilized after contact with the wound. These materials are used to fill the wound cavity and when placed in contact with the wound they present a smooth, hydrophilic porous surface. Excess water (exudates composed of wound fluids) and cell debris are absorbed and retained inside the foam. In addition, foams protect the wound from excess pressure. Water vapours and gas transmission can be adjusted by varying the volume fraction of polymer and crosslink density. The one disadvantage of a foam is the low tensile strength and lack of structural integrity [burnsurgery.org].

Examples: Laminated polyurethane foam, Nonlaminate, non-adherent foam (polyurethane), Closed-cell polyurethane foam, Silastic foam, Poly(ε-caprolactone) foam (covered with polyurethane film)

e) Composite Dressings
These are composed of laminates of two or more layers. The outer layer is designed for durability and elasticity and may serve as a rate controller for water evaporation, while the inner layer is designed for maximum adherence and elasticity. Composite dressings may be classified as follows:

i. Hydrocolloid based Dressings: These dressings are compound formulations containing a cocktail of elastomeric adhesive and gelling agents. Carboxy methyl cellulose is the most common absorptive ingredient acting as absorbent for wound fluid [worldwidewounds.com].

ii. Hydrogel Sheets: These are sheets of 3-D networks of cross linked hydrophilic polymers. They swell with aqueous solutions and act as absorbent. The most commonly used polymers are polyethylene oxide, polyacrylamide and polyvinylpyrolidone. Owing to their unique cooling ability, they are of great benefit for use as a first aid measure for thermal burns.

iii. Hydrogel Amorphous: These are similar in composition to sheet hydrogels except that the polymer has not been cross linked to form a sheet. They contain small quantities of collagen, alginate or complex carbohydrates. They are unique in their ability to donate moisture to a dry wound eschar and facilitate autolytic debridement in wounds [Willi P, et.al; 2000]. But owing to the viscosity of the amorphous hydrogel, it may be difficult to
retain it in the wound bed. However, they exhibit more rapid rate of closure and re-
epithelialization as compared with the hydrocolloid wound dressings.

1.1.6 Next generation products for wound management
Normal wound healing requires both restoration of cover by re-epithelialisation, and
restoration of support by ingress of collagen. The first occurs by migration and
proliferation of keratinocytes from the wound edges and by differentiation of stem cells
from remaining hair follicle bulbs. The second occurs by influx of growth factors
secreted by macrophages, platelets and fibroblasts, by fibroblast proliferation and
subsequent synthesis and remodelling of collagenous dermal matrix. However, in the
case of full-thickness acute burn injuries and chronic wounds (pressure ulcers, venous
ulcers and diabetic foot ulcers), these processes are defective and new technologies are
being developed to improve the healing in these conditions [worldwidewounds.com].
The importance of formal testing of new dressings against traditional methods, prior to
their use in clinical practice, was recently emphasized [Price RD, et.al; 2001, Harding
KG, et.al; 2001].
Some of the next generation products are described in this section.

1.1.6.1 Bioactive dressings
Antimicrobials
An important consideration in the design of new dressings is their ability to combat
microbial infection. Many dressings now exploit 'bioactive' properties to promote healing
and control infection [Moore K, et.al; 1997]. These include the now well-known
sustained release iodine and silver dressings (e.g. Iodosorb, Actisorb Silver 220).
Actisorb Plus is an activated charcoal cloth impregnated with silver. It is reported to
absorb bacteria, which are then inactivated by the silver [Furr JR, et.al; 1994].

![Actisorb silver 220 (J&J)](image1)

![Iodosorb (Smith & Nephew)](image2)

Now marketed as Actisorb Silver 220, it is intended for use over partial or full thickness
wounds such as pressure ulcers, venous ulcers, diabetic ulcers and acute and chronic
wounds [Leaper D, et.al; 2006], and is claimed to be the only dressing currently available that 'combines broad-spectrum antimicrobial action, bacterial toxin management and odour control' (J+J news, March 2003).

Another new generation product, Acticoat, utilises novel silver-coating technologies in a dressing designed to prevent wound adhesion, control bacterial growth and facilitate burn wound care. It consists of a rayon/polyester non-woven core, laminated between layers of silver-coated high-density polyethylene mesh. It is claimed by the manufacturers to provide an effective antimicrobial barrier for up to 3-5 days against 150 pathogens, including both Methicillin Resistant Staphylococcus aureus (MRSA) and Vancomycin-resistant Enterococci (VRE).

*Acticoat*

**Interactive dressings**

Exploitation of the bioactive properties of dressings is not confined to antimicrobials, but is becoming more commonplace with the increase in use of alginates, hydrocolloids, and materials containing collagen or other extracellular matrix components (in particular hyaluronic acid). As well as maintaining a moist wound environment, these dressings are believed to interact with cells or matrix proteins in the wound bed to promote healing. Alginates are highly absorbable biodegradable dressings derived from seaweed (e.g. Kaltostat, Tegagen, SorbSan, SeaSorb, Algisite M, Algosteril). They contain building blocks of mannuronic acid (M) and guluronic acid (G) building blocks: the high-M alginates are soft and gel-like, while the high G alginates are more stable and are ribbon or rope-like [Furr JR, et.al; 1994]. Large quantities of alginates are used each year to treat exudating wounds such as leg ulcers, pressure sores and infected surgical wounds. As well as controlling exudate by ion exchange, alginates are believed to exert a bioactive effect by activating macrophages within the chronic wound bed to generate pro-inflammatory signals (such as tumour necrosis factor (TNF)-alpha, interleukin (IL)-1, -6 and -12). This may then initiate a resolving inflammatory response characteristic of
healing wounds [Skjak BG, et.al; 2000]. It is now well known that chronic wounds are characterized by a macrophage rich inflammation [Moore K, et al. 1997] and any putative macrophage defect probably relates to the functional status of the macrophages present at the wound site [Wysocki AB, et.al; 1993].

Promogran is a sterile, freeze-dried matrix made up of collagen and oxidized regenerated cellulose (ORC). This treatment is recommended for use on all types of chronic wounds that are free of necrotic tissue and show no clinical signs of infection. Once in place it must be covered with a low-adherent secondary dressing to maintain a moist wound-healing environment. It can be used in conjunction with standard compression therapy and need only be changed as clinically required [Cullen B, et.al; 2002].

Tissue-engineered 'skin equivalents'
Surgical grafting of split-thickness autologous skin is the standard method for rapid closure of full-thickness burn wounds. However, advances in cell culture techniques have involved the development of autologous and allogeneic grafts using either sheet of fibroblasts in a biodegradable matrix or cultured keratinocyte sheets. It is established that superior results are obtained if both dermal and epidermal components are combined, for example in a bilayer skin equivalent. The requirement of basement membrane proteins and the importance of dermal-epidermal interactions have been highlighted. Design principles for cultured skin substitutes have recently been examined, as has the
theoretical potential of their use on burns. A clinical evaluation of skin substitutes has also been reported [Boyce ST; 2001, Falanga V, et.al; 1998, Falanga V, et.al; 1999, Leigh IM, et. al; 1987, Kangesu T, et.al; 1993].

**Cell-free matrices**

Two approaches are currently used in the production of cell-free dermal matrices. The first is a synthetic matrix, comprising of collagen and other extracellular matrix components, that attempts to recreate the desired physical and chemical properties of the dermis. One example now in clinical use is Integra artificial skin, developed by Burke and Yannas in the early 1980s [Shakespeare P; 2001]. The second approach is the use of native dermis, from which the cellular components have been removed. This may be treated to preserve the dermal architecture (e.g. Alloderm).

Integra (a dermal regeneration template) by Integra life sciences comprises a porous collagen/chondroitin-6 sulphate matrix overlaid with a thin silastic sheet, which acts as a scaffold for dermal regeneration. Its unique action essentially inhibits granulation and promotes the growth of neo-dermis through the collagen and glycosaminoglycan matrix. The silastic layer provides a temporary epithelial covering, which is removed prior to secondary grafting with a thin split-thickness autograft or cultured keratinocyte sheet.

Cell-containing matrices

As with cell-free matrices, cell-containing matrices include both synthetic matrices, often made of polyglycolic acid mesh, composed of human fibroblasts (e.g. Dermagraft), as well as natural biological substrates usually comprising collagen and glycosaminoglycans (e.g. Apligraf).

Alternatively, non-cellular matrices, such as the hyaluronic acid scaffolds, (e.g. Hyalograft-3D, Laserskin) are recommended for culture with autologous patient cells prior to grafting [Kearney JN, et.al; 2001].
Apligraf (originally called Graftskin) is recommended for use on venous ulcers and is the only bilayered living skin equivalent currently approved by the FDA. Hyalograft 3D and Laserskin (Hyaff-11) are indicated for use on diabetic foot ulcers and venous leg ulcers. Comprised entirely of a benzyl ester derivative of hyaluronic acid, they may be used as scaffolds for the cultivation of fibroblasts and keratinocytes [Burke JF, et al; 1981].

Cost versus effectiveness of new treatment regimens
What most studies fail to address, despite being a major determinant factor in treatment, is cost. The cost-effectiveness of new treatments in comparison to standard care must be considered, not only in terms of direct treatment costs, but also in terms of length of initial hospital stay, requirements for home care, additional bandaging regimens, and quality of the overall outcome.

Whilst the new treatment regimens initially may be perceived to be more expensive than traditional treatments, in many cases this additional cost is justifiable. With respect to novel dressing types, considerable clinical experience in the Wound Healing Research Unit at Cardiff has indicated that not only are some of the new treatments cost effective, but they have also proven to be extremely beneficial in terms of their ability to reduce pain, odour and leakage from the wounds [Lam PK, et.al;1999].

The above literature survey indicated the necessity and availability of advanced wound management products. Hence, attempts were made to design and develop following
types of advanced wound management products during this research project to provide cost effective advanced wound management.

1. Absorbable haemostats.
2. Haemostats to prevent postoperative infection.
3. Haemostats to provide postoperative analgesia.
4. Hydrogels for moist wound healing.
5. Hydrogels for antibacterial wound healing.
6. Hydrogels for moist wound healing with anesthetic effect.

A detailed account of above types of wound dressings is presented in the following sections 1.2 and 1.3.
1.2 Absorbable hemostats

The control of bleeding is a serious problem in certain surgical procedures and in various types of emergency wounds. Bleeding from the kidney, brain, or liver or the persistent oozing from severed capillaries and veins is particularly difficult to control by conventional means such as suturing or ligature and in many cases is serious enough to endanger life. Surgical hemostats consisting of conventional gauze pads or similar articles impregnated with a haemostatic materials such as ferric chloride, thrombin or the like, have been used for many years to arrest bleeding. However, haemostats of this type cannot be left in situ in a closed wound since foreign body tissue reaction would result. This is a serious disadvantage. The haemostat at the bleeding site may frequently also disrupt any blood clot which has formed and cause renewed bleeding [Larson PO; 1988].

An ideal haemostatic agent should be such that the agent itself as well as any of its metabolic breakdown products would be safe to use within the body, it should be efficacious and easy to use in a variety of different circumstances. Again the main property is affordability and cost [Collins JA, et al; 1969].

Gelatin sponges facilitate clot formation and reduce blood loss. The first haemostatic agent used in this capacity was based on fibrin foam, derived from blood plasma. After World War II, the shortage of blood donations lead to scarcity of fibrin and sparked the drive to find an alternative. Pharmacia, an USA based company developed a haemostatic agent made from specially treated porcine derived gelatin, called Gelfoam, which proved to be as effective as fibrin foam[Correll JT, et.al; 1954, BP 1993].

Gelatin sponge or Gelfoam, which is also known commercially as Surgifoam was first introduced in the 1940s by Dr. Gray in the neurosurgical procedures. It is prepared from purified pork skin gelatin [Pharmacia & Upjohn Gelfoam Brochure; 1996, 1997]

Oxidized regenerated cellulose is also known as Surgicel or Oxycel in its commercial forms. It is derived from alpha-cellulose that is actually plant-based. Surgicel comes in knit form, whereas Oxycel comes in a microfibrillar form. Surgicel is relatively acidic
Introduction

Design and development of surgical dressings for advanced wound management

and is thought to cause some small vessel contraction. Like Gelfoam, it works at the same point in the intrinsic pathway of clotting causing contact activation. So again the same thing holds that functional clotting factors are needed in order for this to work. It is relatively more bacteriostatic when compared to other haemostatic agents. The theory behind this is that because of its relatively low pH, it deactivates and denatures some of the bacterial proteins especially those related to antibiotic resistance, thus making them more susceptible to antibiotics. It needs to be applied dry and gets absorbed within four to eight weeks [Pharmacia & Upjohn Gelfoam Brochure; 1996, 1997].

Surgicel (J&J)

Microfibrillar collagen commercially known as Avitene is most commonly used in a light flour form, but it also comes in a non-woven web form. This is collagen, which is derived from bovine skin. It binds tightly to blood surfaces. It causes minimal swelling especially when compared to Gelfoam. The way it works is slightly different because in addition to being collagen and causing contact activation, it directly activates platelets. But again, it works very proximally within the intrinsic pathway. It is absorbed in three months and it needs to be applied dry [Morgenstern L; 1974].

Collagen sponges come in a wide variety of different commercial forms. Again they are similar to Avitene and derived from bovine Achilles tendon or bovine skin and it works in exactly the same way as Avitene and are absorbed in 8-10 weeks [Hait MR; 1970].

The next class of haemostatic agents is slightly different which is called as topical thrombin. The idea of topical thrombin has been around since the early 1900’s in order to try to achieve clot. In 1999 a new agent was introduced called Floseal [Alexander JM, et.al; 1978] which consists of bovine thrombin plus cross-linked gelatin granules mixed together. So it works like bovine thrombin by directly activating fibrinogen and converting it into fibrin monomers. It works down within the clotting cascade in the common pathway bypassing all the other necessary clotting factors. Person should have functional fibrinogen in order for this to work. The product Floseal itself is slightly different from topical thrombin plus Gelfoam because the gelatin granules have been
cross linked in such a way that they do not swell to same extent. It is absorbed in approximately 6-8 weeks [Rondinone JF, et.al; 2004].

Fibrin sealants commercially come in many forms including Tisseal and Crosseal and there are many variations of fibrin sealants. In some cases, pure fibrinogen is combined with bovine thrombin and thrown in an antifibrinolytic agent and mixed well. So it works like the bovine thrombin. It converts this exogenous human fibrinogen to fibrin monomers, but the patient’s own factor XIII and calcium are required which then converts it into fibrin polymer. They usually add an antifibrinolytic agent to the mix as well in order to stabilize the clot. They are absorbed within 10-14 days [Schoenecker JG, et.al; 2003].

There are some completely autologous fibrin sealants. The patient’s own serum is taken and the fibrinogen and thrombin are purified. This achieves essentially the same effect as the fibrin sealants. There are Target Platelet Gels where again platelets are purified with plasma and the patient’s own serum is combined with thrombin to get similar product as fibrin sealants. Only there are some additional benefits, some platelet growth factors are directly involved which help in wound healing. There are also some completely synthetic agents available, which are made from polyethylene glycol gels when combined, they make a completely synthetic hydrogel [Rousou JA, et.al; 1984].

Some of the products which are used as haemostatic are given in table 1.2.
Table 1.2 Examples of haemostatic agents

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Haemostatic agent</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CROSSEAL Fibrin Sealant (Human)</td>
<td>For patients undergoing liver surgery as an effective haemostatic agent for controlling active bleeding.</td>
</tr>
<tr>
<td>2.</td>
<td>SURGICEL FIBRILLAR Absorbable Haemostat</td>
<td>For optimal adherence and conformability to bleeding sites.</td>
</tr>
<tr>
<td>3.</td>
<td>SURGIFOAM Absorbable Gelatin Sponge, USP</td>
<td>Unsurpassed Performance in a Gelatin Sponge.</td>
</tr>
<tr>
<td>4.</td>
<td>SURGIFOAM Absorbable Gelatin Powder</td>
<td>For haemostasis in hard-to-reach places.</td>
</tr>
<tr>
<td>5.</td>
<td>SURGIFOAM Absorbable Gelatin Powder Kit</td>
<td>Customized consistency for rapid haemostasis.</td>
</tr>
<tr>
<td>6.</td>
<td>INSTAT Collagen Absorbable Hemostat</td>
<td>Utilizing Collagen to Achieve Superior haemostasis.</td>
</tr>
<tr>
<td>7.</td>
<td>SURGICEL NU-KNIT Absorbable Hemostat</td>
<td>Excellent strength and coverage for heavier bleeding.</td>
</tr>
<tr>
<td>8.</td>
<td>SURGIFLO Haemostatic Matrix</td>
<td>Enhanced contact. Advanced control.</td>
</tr>
</tbody>
</table>

These products are manufactured by Johnson and Johnson Company and are distributed in international market. Only Surgifoam gelatin sponge is distributed by Ethicon in India. The absorbable haemostatic agents and dressings contain porcine or bovine gelatin, bovine collagen, or regenerated oxidized cellulose. The two most recently approved absorbable haemostatic agents and dressings, FloSeal and CoStasis, additionally contain bovine thrombin and therefore are combination products, i.e., products containing both a device and biological component [Chang H, et.al; 1992, Wagner WR, et.al; 1996].

The key players in Gelatin sponge industry are Ferrosan, (whose product is distributed by J&J Ethicon), B Braun and Pharmacia Upjohn. Ferrosan and Pharmacia use porcine gelatin. B Braun has two products, one for dental application based on Porcine gelatin and a second based on Bovine gelatin for surgical procedures [Hong SR, et.al; 2001, Barbolt, et.al; 2001].

Gelatin sponges may be used with success in practically all forms of surgery. It is particularly advantageous where venous or diffuse oozing haemorrhage cannot be controlled by conventional means. These can be used in Parenchymal surgery, Thoracic surgery, Mediastinoscopy, Vascular surgery, Plastic surgery, Neurosurgery, Dermatology and Otorhinolaryngology.
Dental sponges are beneficial after tooth extractions. They provide an effective haemostatic tool for dentists and oral surgeons, for use when conventional haemostasis is difficult during tooth extractions and other surgical procedures. These sponges are inserted into the alveolus if oozing bleeding persists or if an arterial bleeding occurs after tooth extractions. For other oral surgical procedures, the sponges are used in case of venous and oozing bleeding [Barbolt, et.al; 2001].

Rapid blood loss from relatively large surfaces is particularly difficult to control since it cannot be blocked by sutures or other ligation means. Attempts have been made to develop a haemostatic sponge which provides a fast and effective composition for inducing rapid blood coagulation and haemostasis at a wound or bleeding site. One such haemostatic sponge composition is an absorbable gelatin sponge. The spongy physical properties of the gelatin sponge hasten clot formation and provide structural support for the forming clot.

1.2.1 Mode of action of absorbable gelatin sponge

Mode of action of absorbable gelatin sponge is not fully understood, it is currently believed that its effect is to be linked to the ability of the gelatin sponge to absorb and hold within its interstices, many times its weight of blood and other fluids. Caught blood platelets interact with the sponge and get activated leading to the formation of a haemostatic plug and cessation of bleeding. This haemostatic plug resembles the natural plug that usually forms in the body after injury. The activated platelets also initiate the coagulation cascade that ends with conversion of soluble fibrinogen into a net of insoluble fibrin by the action of thrombin. Factor XIII which is activated by thrombin in the presence of Ca$^{2+}$ cross-links and stabilizes the clot's fibrin monomers [Correll JT, et.al; 1945].

1.2.2 Medicated absorbable gelatin sponges

GELFOAM® and SURGIFOAM® are an example of haemostatic devices which can be applied dry or moistened with sterile saline or thrombin directly to the wounded site to obtain control of the bleeding. In order to enhance the natural haemostatic property of gelatin, products or kits that combine the haemostatic features of gelatin sponge, thrombin and Ca$^{2+}$ have been developed and manufactured. For example, it is customary that in surgery the gelatin sponge is removed from its package, dipped into diluted thrombin solution and kneaded vigorously until all air is expelled. This step is followed...
by a second immersion in thrombin solution and application of the wet sponge to the bleeding organ with light pressure. However, the soaking of the sponge requires time-consuming and cumbersome procedures, including thawing and predilution of the concentrated thrombin solution. Each of the preparation steps introduces potential errors which might compromise the sterile preparation and vary the efficacy of the sponge. Moreover, the complicated procedure requires administration of the sponge by trained emergency personnel. Another major drawback in the technique is that a large volume of liquid is required to fill the sponge voids consequently resulting in a low concentration of thrombin and Ca^{2+} at the interface between the sponge and the injured site. As a result, the sponges are ineffective in providing and maintaining haemostasis. To overcome this problem, it is preferable to have required drug loaded into the haemostatic sponge. The following publications disclose coating of a cross-linked gelatin sponge with a solution of an active ingredient and drying the sponge.

US patent 5,643,596 and Patent WO9512371 discloses a haemostatic patch comprising a matrix such as absorbable gelatin sponge and an effective amount of e-aminocaproic acid (EACA) on only one side of the matrix. According to the description the matrix can be coated before and after addition of EACA with thrombin solution. The EACA can be applied by spraying powder, by coating a solution onto the matrix, or by complete or partial dipping. Drying of the wetted sponge is accomplished preferably by lyophilization. The patent application emphasizes the importance of EACA in the patch and is silent on a biodegradable matrix without EACA [Israel N, et.al; Patent WO/2009/109963.2009].

WO9013320 patent relates to a haemostatic sponge comprising a porous structure of biologically absorbable solid material such as denatured gelatin sponge, thrombin, and one or more thrombin stabilizing agents. The haemostatic sponge is prepared by introducing into the sponge by injection at a multiplicity of sites an aqueous solution of thrombin. The injection is carried out without resulting in leakage of the injected liquid to the surfaces of the sponge material. The sponge is then air-dried at a temperature of 30-100°C for time period sufficient to reduce the water content to below 50%. According to the description, the injection of the thrombin solution may result in structural deformation of the sponge [Israel N, et.al; Patent WO/2009/109963.2009].
Introduction

US Patent 2,558,395 discloses a ready-to-use gelatin sponge containing thrombin. According to the patent, thrombin is added to an aqueous gelatin solution, transformed into foam and dried in vacuum at low temperature. The gelatin in this patent was not cross-linked at any stage during the preparation. Thus, upon contact with blood, the gelatin component is dissolved, the thrombin is released immediately and causes the transformation of fibrinogen to fibrin and a fibrin film is formed over the wound [Israel N, et.al; Patent WO/2009/109963.2009].

US Patent 4,292,972 relates to a lyophilized foam sponge product which has a hydrocolloid composition. According to the description the solubility and absorbability of the lyophilized foam product can be reduced by cross-linking either before or after the lyophilization procedure. The lyophilized foam product is formed from a mixture of gelatin, pectin and sodium carboxymethylcellulose [Israel N, et.al; Patent WO/2009/109963.2009].

US Patent 4,265,233 discloses a wound healing material to which factor XIII with or without thrombin has been fixed by covalent bonding, ionic bonding adsorption or entrapping. According to the description the wound healing material may be synthetic or natural polymers. The patent discloses several natural occurring proteins, including cellulose, viscose rayon, cupraammonium rayon, cellulose acetate, carboxymethyl cellulose, methyl cellulose, agarose, dextran, pullulan, pectin, alginic acid, chitin, polysaccharides such as mucopolysaccharides and proteins such as wool, silk, collagen, gelatin and casein. The examples disclose dipping of a gelatin sponge in the size of 5 x 2.5 x 0.5 cm in 10 ml of an aqueous solution of factor XIII with or without thrombin and subsequent freeze-drying for 20 hours.

Patent EP0277096 discloses haemostatic materials, such as GELFOAM®, SURGICEL®, and AVICEL®, and collagen which can be effectively used in combination with a stabilized thrombin formulation. According to the patent, the preparation must contain polyols and at least one buffer such as acetate or phosphate buffer. According to the description the stabilized solution is preferably absorbed onto the haemostatic agent and the pad is freeze-dried and packaged in a sterile manner [Israel N, et.al; Patent WO/2009/109963.2009].
The patent WO 02072128 discloses a cross-linked gelatin composition which has a wetting agent incorporated therein. According to the description the wetting agents can be coated over the surface of the gelatin sponge. The examples show that addition of the wetting agent onto the surface of the sponge is carried out by placing the sponge into a vial containing a solution of a wetting agent and a solvent. The vial is then inverted to allow the solution to soak into the sponge. The coated composition is then removed, drained of excess liquid and air dried overnight. According to the description the gelatin composition may also include a medicament such as thrombin, fibrinogen, factor XIII and other coagulation factors [Israel N, et.al; Patent WO/2009/109963.2009].

The patent EP0568334 relates to a collagen-containing sponge comprising an absorbable gelatin sponge, collagen, and an active ingredient. The absorbable gelatin sponge can be combined with the collagen and the active ingredient by transferring a predetermined amount of a collagen solution on top of the gelatin sponge. The example discloses a preparation of a collagen sponge by pipetting 0.24 or 0.4 ml of collagen solution containing platelet-derived growth factor (PDGF) on top of a 1 mm gelatin sheet. Following soaking, the sponge is dried, preferably, at room temperature for a period of about an hour to five days. It is indicated in the patent that in order to improve flexibility of the sponge a suitable plasticizer can be used [Israel N, et.al; Patent WO/2009/109963.2009].

The patent WO9306855 relates to a haemostatic composition comprising a haemostatically effective amount of factor VIIa together with a biologically compatible carrier such as a biodegradable sponge material. The carrier does not contain thrombin or any other blood clotting factor. The description discloses several materials for preparation of haemostatic sponges such as collagen, gelatin such as denatured gelatin, chitin, cellulose, polyglycolic acid and polyacetic acid. The sponge may be prepared by saturating a preformed dried sponge with a solution of FVIIa followed by freeze-drying. The examples disclose soaking of 5 mm cores of gelatin sponge in 2 ml of sterile water which contained factor VIIa. The wet sponge was applied to the bleeding site without drying [Israel N, et.al; Patent WO/2009/109963.2009].

The patent US2558395 describes gelatin sponges containing thrombin as an additional hemostatic agent. The thrombin is added to an aqueous solution of gelatin, which is then freeze-dried to form a soluble gelatin sponge containing thrombin. The patent

1.2.3 Development of Plain/Medicated Absorbable gelatin sponges

Gelatin sponges are made by whipping a solution of gelatin and drying the foam, usually by lyophilization. Unlike collagen which is naturally insoluble in aqueous neutral solutions, gelatin is soluble at temperatures above 30°C, especially at 37°C, the physiological temperature. This characteristic renders the sponge unsuitable for in vivo use as the sponge would dissolve quickly and lose its structural integrity and porous structure. The gelatin must therefore be cross-linked in order to prevent its rapid dissolution in the blood. Methods of cross linking include treatment of the sponge with a chemical cross-linking agent such as formaldehyde, glutaraldehyde, and carbodiimides (e.g. EDC) or via treatment of the dry sponge with dry heat (100-160°C for several hours) [Nakayama, et.al; 1995].

Lyophilization was preferred method for formulation of absorbable gelatin sponges.


Gelatin sponge can be developed by (a) dispersing gelatin in a solvent to form a gelatin dispersion; (b) drying the gelatin dispersion to produce a gelatin sponge (c) crosslinking the gelatin to render it substantially insoluble in water followed by (d) irradiating the sponge with gamma radiation. The inventors found that irradiation of the sponge can be used to prepare a gelatin sponge that breaks down more rapidly in the presence of collagenase, without loss of hemostatic properties.

The crosslinked gelatin sponge is preferably made by the following process. Gelatin is dispersed in an aqueous solvent, like water, at a concentration of about 1 to 10%. A gas such as filtered air is blown through the solution to produce thick foam. The foam is then shaped by spreading onto plates, before drying.
The drying is carried out by freeze drying, and/or by evaporation at elevated temperatures. These methods of forming the sponges are described in more detail in US-A-2558395 and WO-A-9527517 patents.

The step of preparing the gelatin sponge further comprises the step of crosslinking the gelatin. The step of crosslinking may be carried out before, during and/or after the step of drying the dispersion. The crosslinking may be carried out by the addition of a chemical crosslinking agent such as dicyclohexyl carbodiimide or glutaraldehyde. The chemical crosslinking agent can be added to the dispersion of the gelatin, or it can be applied to the gelatin after drying.

The moisture content of the gelatin sponge before, during and after irradiation is preferably less than about 10% by weight. The sponge may be cast in any convenient shape, including tubes and sheets for use in surgery. In other embodiments of the method according to the invention, the sponge may be comminuted into smaller pieces, or into a powder, suitable for spreading over a wound to arrest bleeding.

The gelatin foams or sponges may be medicated by the addition of one or more therapeutic agents before or after the drying step. In the case of post-drying treatment, the inventors found that treating the sponges with therapeutic agents dispersed in water or methanol causes the sponges to collapse. Preferred alcohols include ethanol, propanol or isopropanol. It has also been found that treating the sponges with the specified alcohols, without adding any therapeutic agent, can render the sponges antimicrobial [Israel N, et.al; Patent WO/2009/109963.2009].

The therapeutic agent is suitably selected from the group consisting of a reactive oxygen scavenger, an antimicrobial agent, an antioxidant dyestuff, a pain relieving agent, a growth factor or mixtures thereof. The reactive oxygen scavenger may be selected from the group consisting of antioxidant phenol derivatives, vitamin E, methyl peroxide antioxidants, stilbenes, gallocatechins, ubiquinol, retinoids, vitamin A, vitamin C, N-acetyl cysteine, selenium and its compounds, zinc and its compounds, glutathione, carotenoids, papain, thioproline, albumin, chlorophyllin [Israel N, et.al; Patent WO/2009/109963.2009].

The antimicrobial agent may be selected from the group consisting of antiseptics and
antibiotics and mixtures thereof. Suitable antibiotics include peptide antimicrobials (e.g. defensins, magainin or synthetic derivatives of them); antibiotics such as gentamicin, tetracycline, penicillins, terramycins, erythromycin, bacitracin, neomycin, polymycin B. mupirocin, clindamycin and mixtures thereof. Suitable antiseptics include silver sulfadiazine, chlorhexidine, povidone iodine, triclosan, other silver salts and colloidal silver, sucralfate, quaternary ammonium salts. Suitably, the antimicrobial agent may be incorporated into the sponges at concentrations of between about 0.1-30% by weight, more suitable from about 0.5-15% by weight, and preferably between about 1-5% by weight based on the weight of the product material [Israel N, et.al; Patent WO/2009/109963.2009].

The pain relieving agent may be selected from the group consisting of an anaesthetic, an analgesic, an anti-inflammatory or mixtures thereof. Suitable anaesthetics include lidocaine or novocaine. Suitable analgesics include non-steroidal anti-inflammatory drugs (NSAIDs). Suitable anti-inflammatory agents include steroids such as prostaglandins. Suitably, the pain relieving agent may be incorporated into the sponges at concentrations of between about 0.1-30% by weight [Israel N, et.al; Patent WO/2009/109963.2009].

The growth factor may be selected from the group consisting of platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF), and mixtures thereof. Suitably, the growth factor may be incorporated into the sponges at concentrations of between about 1ppm to about 1% the weight of the product material [Israel N, et.al; Patent WO/2009/109963.2009].

The methods of preparing medicated gelatin sponges comprise soaking the sponge in the alcohol solution of the therapeutic agent so that the whole volume of the sponge is wetted by the solution, followed by draining of the sponge and drying the sponge under mild conditions or under reduced pressure. In other embodiments, the sponge may be sprayed or otherwise surface coated with the alcohol solution of the therapeutic agent so that only a surface region of the sponge contains the therapeutic agent.

The step of irradiation with ionizing radiation is carried out with gamma radiation. Based on literature survey and available facilities, attempt was made in this project to develop medicated gelatin sponges using Gentamicin sulphate and Lidocaine hydrochloride as drug candidates having antimicrobial and pain relieving activity respectively.
1.3 Preparation of absorbable gelatin sponges

Based on literature review, lyophilization is the best process to be used for preparation of uniformly porous, biodegradable gelatin sponges having high water absorbing capacity.

1.3.1 Lyophilization

Lyophilization which is also termed as freeze drying is the preferred method for preparation of absorbable gelatin sponges. Biological materials are dried to stabilize them for storage or distribution. Drying always causes some loss of activity or other damage. Lyophilization is a method of drying that significantly reduces such damage. Because lyophilization is the most complex and expensive form of drying, its use is usually restricted to delicate, heat-sensitive materials of high value. Substances that are not damaged by freezing can be lyophilized. They rehydrate easily and quickly because of the porous structure left after the ice has sublimed. Specialized equipment is required to create the conditions conducive to the freeze drying process. The equipment can accommodate freeze drying of materials from laboratory scale projects to industrial production [Pikal MJ; 2004].

Properly freeze dried products do not need refrigeration, and can be stored at ambient temperatures. Because the cost of the specialized equipment required for freeze drying can be substantial, the process may be an expensive undertaking. However, savings realized by stabilizing an otherwise unstable product at ambient temperatures, thus eliminating the need for refrigeration, more than compensate for the investment in freeze drying equipment.

Desired characteristics of a lyophilized product

A lyophilized product should possess certain desirable characteristics, including

● long-term stability
● short reconstitution time
● elegant cake appearance
● maintenance of the characteristics of the original dosage form upon reconstitution, including structure or conformation of proteins; and particle-size distribution.
1.3.2 The lyophilization process

The lyophilization process consists of three stages: freezing, primary drying and secondary drying [Frank KB, et.al; 2004].

**Freezing**

During this stage the formulation is cooled. Pure crystalline ice forms from the liquid, thereby resulting in a freeze concentration of the remainder of the liquid to a more viscous state that inhibits further crystallization. Ultimately, this highly concentrated and viscous solution solidifies, yielding an amorphous, crystalline, or combined amorphous–crystalline phase.

**Primary drying**

The ice formed during freezing is removed by sublimation at subambient temperatures under vacuum. This step traditionally is carried out at chamber pressures of 40–400 Torr and shelf temperatures ranging from -30°C to -10°C. Throughout this stage, the product is maintained in the solid state below the collapse temperature of the product in order to dry the product with retention of the structure established in the freezing step.

The collapse temperature is the glass transition temperature ($T_g$) in the case of amorphous products or the eutectic temperature ($T_e$) for crystalline products.

**Secondary drying**

The relatively small amount of bound water remaining in the matrix is removed by desorption. During this stage, the temperature of the shelf and product are increased to promote adequate desorption rates and achieve the desired residual moisture.

![Figure 1.6 Freeze drying cycle](image)

*Figure 1.6 Freeze drying cycle*

[F: freezing; PD: primary drying and SD: secondary drying]
1.3.3 Possible destabilizing effects of the Lyophilization process

**Freezing**

Freezing damage can occur with labile products such as proteins [Pikal MJ, et.al; 2004]. Initial ice-crystal size depends on the relative contributions of nucleation and crystal growth of ice. A rapid nucleation and growth rate resulting from a large degree of supercooling leads to a larger number of small ice crystals, which in turn presents a large ice–water interface [Searles JA, et.al; 2001]. Exposure of proteins to this ice–water interface can lead to denaturation.

The freezing step will determine the structure of the final dried cake as well as the drying rate. Small ice crystals produce pores with lower volume–surface area, thus resulting in lower diffusive flux and slower sublimation rates [Searles JA, et.al; 2001].

**Drying**

Removal of the hydration shell from proteins during drying in the absence of the appropriate stabilizers can cause destabilization of the protein structure [Crowe JH, et.al; 1993]. Extremely low water content in the final product can result in destabilization, and optimal water content should be determined. The desired residual moisture must be correlated to stability during long-term storage as part of development studies [Shalaev EY, et.al; 1996].

**Glass-transition temperature and its significance**

When heated, sugar glasses undergo a second-order transition from a rigid state to a viscoelastic rubbery state. The temperature at which the vitreous transformation occurs is the glass-transition temperature ($T_g$). When a product exceeds the $T_g$ value, the rigid
glass softens to become a highly viscous rubbery material and collapses. The $T_g$ value of a formulation can be determined by Differential Scanning Calorimetry (DSC), and the collapse temperature is measured by freeze-drying microscopy. Primary drying is always performed at the highest possible temperature while maintaining the product below the collapse temperature.

The dried amorphous product material also has a $T_g$ value. As water is removed during secondary drying, $T_g$ increases. Storage below $T_g$ is important for several products to maintain the rigid-glass structure and hence stability of the product [Duddu SP, et.al; 1997].

**Stability of Freeze Dried Products**

Several factors can affect the stability of freeze dried material. Two of the most important are moisture and oxygen. All freeze dried products have a small amount of moisture remaining in them termed residual moisture. The amount of moisture remaining in the material depends on the nature of the product and the length of secondary drying. Residual moisture values range from <1% to 3% for most products [labconco.com].

Based on literature review and stability consideration of gelatin sponge, process of Lyophilization was attempted for drying of gelatin sponge in this research project so as to get good quality product.
1.4 Hydrogels

Hydrogels by definition are three-dimensional swollen networked structures. Certain materials, when placed in a compatible aqueous medium, are able to swell and retain the volume of the absorbed aqueous medium in their three-dimensional swollen network. Such aqueous gel networks are known as hydrogels or aquagels. Included in this definition are a wide variety of natural materials of plant and animal origins, chemically modified, naturally occurring materials and synthetic polymeric materials [Park K, et.al; 1993].

1.4.1 Synthetic Hydrogels

Synthetic polymeric hydrogels are three-dimensional swollen networks of hydrophilic homopolymers or copolymers covalently or ionically crosslinked [Ratner BD, et.al; 1976]. The original polymeric hydrogel network was developed by Wichterle and Lim in Czechoslovakia in 1954. It was a copolymer of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) for use as contact lenses. Due to lack of interest and support from the appropriate authorities, no success was achieved [Peppas NA, et.al; 1976]. Wichterle and Lim however, continued to work on their development and it was not until the 1960s when the versatility of synthetic polymeric hydrogels was visualised from a commercial point of view [Kost J, et.al; 1987, Mack EJ, et.al; 1987, Park H, et.al; 1996, Ratner BD; 1989].

Polymeric hydrogel networks are formed by various techniques; however the most common synthetic route is the free radical polymerization of vinyl monomers in the presence of a bifunctional crosslinking agent and a swelling agent. The resulting polymer is interesting in the sense that it exhibits both liquid-like and solid-like properties. The liquid-like properties result from the fact that the major constituent (>80%) is water. However, the polymer also exhibits solid-like properties due to the network formed by the crosslinking reaction, or more like elastic solids in the sense that there exists a remembered reference configuration to which the hydrogel returns after being deformed for a long time [Guven O, et.al; 1999].

The classification of hydrogels depends on their physical structure and chemical composition. A common classification, especially useful in biomedical applications includes neutral hydrogels, ionic hydrogels and swollen interpenetrating networks (IPNs) [Peppas NA, et.al; 1986]. The most characteristic property of a hydrogel is that it swells
in the presence of an aqueous media and shrinks in its absence [Park H, et.al; 1996]. In general hydrogels swell to an equilibrium value of 10 – 98 % at physiologic temperature, pH and ionic strength [Ratner BD; 1989]. A dried hydrogel imbibing at least 20 times its own weight of the aqueous media while retaining its original shape is referred to as superabsorbent. The capacity of hydrogels to absorb the aqueous media could be enormous and can be as much as 1000 times the weight of the polymer. Figure 1.8 depicts a hydrogel network upon placement in water [Ratner BD; 1981].

Swelling in hydrogels when in aqueous media occurs in a similar manner as that of an analogous linear polymer dissolving in the media to form a solution [Flory PJ, 1953]. Mainly the nature, predominantly the hydrophilicity / hydrophobicity of polymer chains and the crosslinking density determine the extent of swelling.

Synthetic hydrogels have been a field of extensive research for the past many decades and it still remains a very active area of research today. Hydrogels can be designed with controllable responses as to shrink or expand with changes in external environmental conditions [Stauffer D, et.al; 1982]. The extent of swelling or de-swelling in response to the changes in the external environment of the hydrogel could be so drastic that the phenomenon is referred to as volume collapse or phase transition [Antonietti M, et.al; 1990].

Hydrogels may respond uniquely to changes in external environmental conditions such as ionic strength, electromagnetic radiation, pH, and temperature [Siegel RA, et.al; 1988, Qiu Y, ey.al; 2001, Peppas BL, et.al; 1989, Kuo JH, et.al; 1988] . These conditions could be introduced individually or in combinations and altered as desired. Other important factors such as the type of salt used for the preparation of buffer [Zanina A, et.al; 2001], solvent used as the medium, photoelectric stimulus and external stress [Zanina A et.al; 2002] are also influential on the hydrogel’s performance. These unique properties make
hydrogels excellent candidates in numerous biomedical, pharmaceutical, agricultural and consumer-oriented fields.

1.4.2 Classification of Hydrogels
Polymeric hydrogels are classified in accordance to their monomeric composition based on the method of preparation; some important classes of hydrogels are homopolymeric hydrogels, copolymeric hydrogels and interpenetrating polymeric hydrogels. The hydrogels are classed as either neutral, anionic, cationic or ampholytic based on the presence of ionic charges on the monomer. Hydrogels may be amorphous or semi-crystalline materials based on their physical nature [Young RJ, et.al; 1991, Hiemenz PC; 1984].

Homopolymeric Hydrogels
Homopolymers are polymeric networks derived from a single species of the monomer, which is the basic structural unit. Homopolymers could have crosslinked or uncrosslinked skeletal structure depending on the nature of the monomer and polymerization technique [Jenkins AD, et.al; 1989]. PHEMA hydrogels are among the most widely studied and used of all synthetic hydrogel materials. There are some uncrosslinked homopolymers, which have been of interest to a number of researchers. Poly(N-vinyl-2-pyrrolidinone) (PNVP), poly(acrylamide) (PAM), poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) are classed as uncrosslinked water-soluble homopolymers. PNVP has found useful applications in biomedicine due to its extreme solubility in water and adequate solubility in many other polar and non-polar solvents. PVA is another important class of uncrosslinked homopolymeric material with numerous potential biomedical and agricultural applications when crosslinked [Korsmeyer RW, et.al; 1984, Brazel CS, et.al; 1999]

Copolymeric Hydrogels
Copolymeric hydrogel networks are comprised of two or more different monomer species with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network. The copolymeric hydrogel networks are generally covalently or ionically crosslinked structures, which are not water soluble [Ratner BD; 1989]. A wide range of important copolymeric hydrogels with vast combinations of compatible monomers are available. They include poly(NVP-coHEMA), poly(HEMA-co-MMA) and poly(HEMA-co-AA) [Ende MT, et.al; 1997, Sperling LH; 1981]
**Interpenetrating Polymer Network (IPN) Hydrogels**

IPN, an important class of hydrogel materials, are defined as two independent crosslinked synthetic and/or natural polymer components contained in a network form as shown in Figure 2. A semi-IPN is an IPN where one of the components is a crosslinked polymer while the other component is a non-crosslinked polymer [Carraher CE; 1996].

The two basic synthetic routes to form IPNs are sequential and simultaneous polymerization methods. The formation of an IPN increases the compatibility of the polymer components thus preventing phase separation and allows access to properties that may be hybrids of those of the component macromolecules [Abad LV, et.al;2003, Vischer KB, et.al;1990]. The IPN formed is both, pH and temperature sensitive. Since there is no chemical bonding between the two polymeric components, each component may retain its own property while the proportion of each network can be varied independently thus obtaining the desired combinations of the properties of the two macromolecule components. The mechanical strength of the hydrogel can be improved by using relatively hydrophobic second polymer in the IPN. Furthermore, one or both of the macromolecular networks of the IPN could be made biodegradable [Zhang J, et.al; 2002, Klempner D, et.al; 1994].

![Figure 1.9 Structure of an IPN](image)

A number of IPNs and semi-IPNs based on polysaccharides such as chitosan and its derivatives, PNVP, PVA, poly(ethylene oxide) (PEO), poly(N-isopropyl acrylamide) (PNIPAM), PEG and poly (methacrylic acid) (PMAA) with potential bioapplications as hydrogel materials have been reported[Yao KD, et.al; 1994, Chen SC, et.al; 2004].

**Non-Ionic Hydrogels**

Non-ionic hydrogels are neutral homopolymeric or copolymeric networks, which do not bear any charged groups in their structure. Neutral hydrogels are prepared by various
polymerization techniques. Neutral hydrogels swell to equilibrium when the osmotic pressure of the solvent is balanced with the sub-chain stretching energy. The collapse and swelling of neutral hydrogel networks occur normally as a result of change in the environmental temperature [Risbud MV, et.al; 2000, Seon JK, et.al; 2003, Gupta KC, et.al; 2002].

**Ionic Hydrogels**

Ionic hydrogels or polyelectrolytes are prepared from monomer/s accompanying ionic charges. The charges could be positive or negative thus giving cationic or anionic hydrogels and furthermore, a combination of both positive and negative charges gives an ampholytic macromolecule. Inclusion of charged species in the polymer backbone enhances the stimuli responsive properties, which could be controlled, depending on the nature of the pendent group thus widening its scope of bioapplications as hydrogels [Wang M, et.al; 2001, Ostroha J, et.al; 2004, Şen M, et.al; 1995, Sutani K, et.al; 2002, Dusek K, et.al; 1968, Salamone JC, et.al; 1985]

**Anionic Hydrogels**

Anionic hydrogel networks are either homopolymers of negatively charged acidic or anionic monomers or copolymers of an anionic monomer and a neutral monomer.

However, anionic hydrogels could also be prepared through modification of existing polymeric non-ionic hydrogels such as by the partial hydrolysis of poly (hydroxyl alkyl

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Design and development of surgical dressings for advanced wound management 41
Introduction

Design and development of surgical dressings for advanced wound management

methacrylates) or by the addition of excess polyanions in polyelectrolyte complexes to form anionic hydrogels. Anionic hydrogels exhibit a marked increase in the swelling ratio with increase in the environmental pH [Haudin JM, et.al; 1986].

**Cationic Hydrogels**

Homopolymers of positively charged basic or cationic monomers or copolymers of cationic and neutral monomers form cationic hydrogel networks. Cationic pendant groups in polymer network in the contrary behaviour to anionic pendant give rise to hydrogels, which remain collapsed in the basic environment and swollen in the acidic environment due to the electrostatic repulsion between the positively charged groups [Ofstead RF, et.al; 1989].

![Cationic monomers for hydrogel formation](image.png)

**Polyampholytic Hydrogels**

Polyampholytic hydrogel networks are referred to as macromolecules capable of possessing both positively and negatively charged moieties in the polymer network [Haudin JM, 1986]. The presence of ionic species along the polymer chain has a distinct effect on the solution and solid-state properties of the polyampholytes. The net charges on these materials can be changed to achieve the desired functional property by changing the monomeric composition of the feed mixture [Peppas NA, 1987].

![Acidic/basic monomers for hydrogel formation](image.png)
1.4.3 Hydrogel Network Structures

Flory (1953) states that the polymeric hydrogel network structure have several roles. In an aqueous medium the network may dissociate and take the role of the solute as in the case of some water-soluble hydrogel networks or swell to equilibrium by imbibing the medium in its structure. The physical and other properties of the hydrogels depend on the structures of the polymeric networks [Kost J, et.al; 1987]. To maintain the three-dimensional structures, polymer chains of hydrogels are usually crosslinked chemically or physically. In chemically crosslinked hydrogels, polymer chains are connected by covalent bonds and thus it is difficult to change the shape of such networks. Polymer chains of physical gels are connected through non-covalent bonds, such as Van der Waals interactions, ionic interactions, hydrogen bonding, or hydrophobic interactions [Guven O, et.al; 1999].

![Schematic representations of hydrogel structures](Figure 1.14)

**Amorphous Hydrogel Structures**

Amorphous hydrogels are optically transparent isotropic polymeric networks that contain randomly arranged macromolecular chains as suggested by Flory. The amorphous hydrogel network contains localized ordered structures or non-homogeneous structures. The temperature at which the polymeric network undergoes the transformation from a glassy to a rubbery state is referred to as the glass transition temperature (Tg). The characteristic feature of amorphous polymeric networks is that when exposed to temperature conditions below its Tg value, they pass successfully through the transformation from a rubbery to glassy state without any clear demarcation between the two phases [Flory PJ, et.al; 1953].

**Semi crystalline Hydrogel Structures**

Semi crystalline hydrogel networks are complex mixtures of amorphous and crystalline phases, which contain dense regions of ordered macromolecular chains. The lack of mechanical strength in some conventional crosslinked hydrogel network structures for
certain biomedical applications has led the development of anisotropic semi crystalline polymeric networks which are characterized by the presence of strong covalent bonds along the polymer chain [Nicholson JW; 1997].

Semi crystalline hydrogel networks are produced by heat treatment of noncrystalline hydrogels above their Tg. Crystallization of polymers in polymer-diluents systems is the method of preparing semi crystalline hydrogel networks. In the crystallization process the short chains that are not able to fold are rejected from the crystalline phase and thus they participate in the amorphous phase hence the resultant polymer network contains continuous composition of amorphous and crystalline regions. The tendency of the polymers to crystallize is enhanced by the regularity of structure and polarity [Carraher CE, et.al; 1996, Solomons TWG; 1984, Peppas NA, et.al; 1993, Morishita M, et.al; 2002, Hennink WE et.al; 2002]. Peppas (1987) suggests that when semi crystalline polymer networks are placed in aqueous medium, only the amorphous regions swell and the crystalline regions are not affected by the medium due to their hydrophobic nature thus they play the role of crosslinks in the polymer network.

**Hydrogen Bonded Hydrogel Structures**

Hydrogen bonding is an electrostatic interaction between electronegative atoms such as oxygen, nitrogen, fluorine and chlorine and hydrogen atoms that are covalently bound to similar electronegative atoms [Park K, et.al; 1993]. The strength of the hydrogen bonding (< 10 Kcal/mol), is far weaker than covalent bonding (> 100 Kcal/mol) but still stronger than the Van der Waals interactions (~ 1 Kcal/mol). The formation of multiple hydrogen bonds between two water-soluble macromolecules result in strong intermolecular structures, which are physically crosslinked three dimensional polymeric networks such as IPNs and semi-IPNs [Bettini R, et.al; 1994].

**1.4.4 Mechanisms for Synthesis of Polymeric Hydrogels**

Polymerization reactions based on kinetics can be divided into chain or step polymerization reactions. Step polymerization reactions generally occur between functionally substituted monomers and are characterized by a rapid disappearance of the monomer at an early stage of the reaction and the existence of broad molecular weight distribution in the later stages of the reaction. Chain polymerization, however, involves a three-step process namely: initiation, propagation and termination, thus allowing the monomer concentration to decrease steadily with time. Thus ideally the reaction mixture at any stage of the polymerization reaction contains the monomer and the converted high
polymer. Contrary to step polymerization reactions, longer reaction times in chain polymerization produce high yield polymers but the molecular weight of the polymer is not affected [Nedkov ES, et.al; 1994, Kabanov VY, et.al; 1998, Chien CL, et.al; 2006].

The saturated monomers for hydrogel synthesis react through a chain polymerization process. The characteristic polymerization of hydrogel network begins from the reactive centre initiated by the polymerization source and terminates upon the loss of the reactivity of the radicals. The reactive centres at the initiation stage could be of free radical nature or ionic nature thus promoting free radical or ionic polymerization [Langner R; 1998, Graham NB; 1990].

1.4.4.1 General scheme for polymerization


**Chain Initiation**

A trace quantity of an initiator is required for photopolymerization and thermal polymerization processes to create free radicals for chain initiation. The initiators readily fragment into radicals under the influence of the applied source.

\[ R \xrightarrow{\Delta/\text{hv}} R^* \]

**Scheme 1** Formation of radicals

Some high-energy ionization radiation sources can also generate radicals through electrochemical means without help of initiator. The radicals created react with an unsaturated monomer to create a new species, thus initiating the chain polymerization process.

\[ R^* + \text{H}_2\text{C}==\text{CH}_2 \xrightarrow{\cdot} \text{R}==\text{C}==\text{CH}_2^\cdot \]

**Scheme 2** Chain initiation

For thermal and photo-curable systems the percentage of the initiator may vary with the weight of total resin. High amounts of initiator have adverse effect since increased free radicals can undergo recombination and inhibit the polymerization process.
Chain Propagation
The propagation step involves growth of the polymer chain by rapid sequential addition of monomer to the active center. The reactivity of the propagating radicals is independent of the size or degree of polymerization.

\[
R\text{-}C\text{-}CH_2\cdot + \left[H_2C\equiv CH_2\right]_n \rightarrow R\left[C\text{-}C\text{-}CH_2\cdot\right]_n
\]

Scheme 3 Chain propagation

Chain Termination
The chain polymerization does not continue until all the participating monomers are used up because the free radicals involved are so reactive that they find a variety of ways of losing their radical activity. Thus the polymer chain terminates by disproportionation or combination reactions.

\[
2R\left[C\text{-}C\text{-}CH_2\cdot\right]_n \rightarrow R\left[C\text{-}C\text{-}CH_3\right] + R\left[C\text{-}C\text{-}CH_2\right]
\]

Scheme 4 Chain termination through disproportionation reaction

The disproportionation reaction is characterized by the interaction of two reactive radical species via hydrogen abstraction process leading to the formation of a saturated and an unsaturated compound.

The combination reaction in the termination stage of the polymer chain occurs by the combination of two reactive radical species to form a single bond and one reaction compound.

\[
2R\left[C\text{-}C\text{-}CH_2\cdot\right]_n \rightarrow R\left[C\text{-}C\text{-}C\text{-}C\right]
\]

Scheme 5 Chain termination through combination reaction

1.4.4.2 Nature of the Reactive Radical Species
The nature of the radical species characterizes the type of chain polymerization. The categories of chain polymerization reactions based on the nature of the reactive species are referred to as free radical for non-ionic radical species, cationic and anionic for
cationic and anionic reactive radical species respectively. The presence of reactive ionic centres makes ionic chain polymerizations more monomer specific than free radical polymerization reactions. Furthermore, the propagating ionic centre is accompanied by a counter-ion of opposite charge and termination does not occur by the reaction of two ionic centres since they are of similar charges and thus repel each other.

The polarity of the polymerization solvent and the ability to solvate the counter ion are significant factors in ionic polymerization. Cationic active centres are created by the reaction of an electrophilic monomer in the presence of protonic acids, which serve as initiators. The termination step in cationic polymerization is achieved by either unimolecular arrangement of the ion pair or through chain transfer [Rosiak JM, et. al; 1988].

Anionic chain polymerization begins with active centers created by the reaction of a nucleophilic monomer but there is no inherent termination process, which is a unique property of such polymers. Termination by ion pair arrangement in contrary to cationic polymerization does not occur in anionic polymerization due to its unfavourable requirement to eliminate the hydride ion and furthermore, the alkaline earth metal counter ions used do not have the tendency to combine with the active carbanion thus rendering the polymer molecule active, also referred to as living polymers [Tobita H; 1993]

1.4.4.3 Curing Processes
The polymerization techniques could be carried out using a variety of curing processes such as thermal, redox and radiation methods. Thermal polymerization technique involves the use of heat in the presence of a suitable initiator while the redox method simply involves a reduction-oxidation reaction between the participating species.

Radiations sources commonly utilized by researchers to synthesize polymeric hydrogels include low energy ultraviolet (UV) radiation technique and high-energy ionisation techniques such as gamma radiation and electron beam radiation [Kabanov V, et.al; 1998, Bray JC, et.al; 1973].

Ionizing Radiation Sources
Ionization radiation is a high-energy process involving electronic radiation of moving particles, which carry enough energy to ionize simple molecules either in air or water
and therefore more penetrative. It involves the use of either electron beams from an electron accelerator or gamma radiation from a $^{60}$Co source [Park K, et.al; 1993].

**Electron Beam (EB) Radiation Process**

Electron beam radiation is a high-energy process, which involves artificially accelerated electron beams delivered from several systems with energy ranging from 0.5 to 20 MeV. The EB process is an efficient process, which does not require initiators in the reactive mixture, however the penetration of fast electron is lower than that of gamma radiation [Park K, et.al; 1993].

**Gamma Radiation Process**

Gamma radiation involves emission of $\gamma$-rays by radioactive isotopes and they cover a wide range of energies. The ease of its preparation and fairly long half-life of 5.3 years compared to other present isotopes makes $^{60}$Co, which sources two monochromatic $\gamma$ beams with energies of 1.17 and 1.33 MeV, the most widely used isotope for this purpose. $^{60}$Co is produced by neutron irradiation of normal $^{59}$Co in a nuclear reactor. Gamma radiation technique does not require the inclusion of chemical initiators of any sort. The gamma rays in contrary to electron beam radiation have very high penetrative power and the dose of radiation could be varied from 5 to 100 rad/sec [Park K, et.al; 1993].

**Ultra Violet (UV) Radiation Process**

UV radiation curing technique uses UV rays from a special light source of desired intensity normally in the presence of a photosensitive chemical. This chemical serves as an initiator in the photopolymerization process to form radicals at a wavelength of 360nm at which monomers are not affected. A medium pressure mercury lamp is an electrode type quartz tube filled with an inert gas such as argon or xenon along with small amount of mercury installed with an electrode at either end. The lamp when connected to an appropriate power source, an electrical arc passes between the electrodes vaporizing mercury resulting in the energy emission, which is primarily a white light, infrared and ultraviolet.

The drawback in this curing method is the use of chemical initiators. The photo initiators are seldom consumed fully during the polymerization process. These materials trapped in the polymer matrix tend to leach out when the polymer is in contact with an aqueous medium. The in vivo leaching of additives used during the fabrication of polymers has been cited as the cause of inflammation and eventual rejection of the implanted biomaterial [Peppas NA, et. al; 1986].
1.4.5 Preparation of hydrogels

Hydrogels are prepared by polymerization with different means of curing. Several techniques have been reported for the synthesis of hydrogels. The first approach involves copolymerization/crosslinking of co-monomers using multifunctional co-monomer, which also acts as crosslinking agent. The polymerization reaction is initiated by chemical initiator. The polymerization reaction can be carried out in bulk, in solution, or in suspension. The second method involves crosslinking of linear polymers by irradiation or by chemical compounds [Rosiak JM, et.al; 1993]. The monomers used in the preparation of the ionic polymer network contain an ionizable group, a group that can be ionized, or a group that can undergo a substitution reaction after the polymerization is completed. As a result, hydrogels synthesized contain weakly acidic groups like carboxylic acids, or a weakly basic group like substituted amines, or a strong acidic and basic group like sulfonic acids, and quaternary ammonium compounds. Some of the commonly used crosslinking agents include N, N'-methylenebisacrylamide, divinyl benzene, and ethylene glycol dimethacrylate [Bray JC, et.al; 1973, Peppas NA, et.al; 1976].

Solution polymerization/crosslinking

In solution co-polymerization/crosslinking reactions, ionic or neutral monomers are mixed with the multifunctional crosslinking agent. The polymerization is initiated thermally, by UV-light, or by redox initiator system. The presence of solvent serves as heat sink, and minimizes temperature control problems. The prepared hydrogels need to be washed with distilled water to remove the unreacted monomers, crosslinking agent, and the initiator. The best example is preparation of poly(2-hydroxyethyl methacrylate) hydrogels from hydroxyethyl methacrylate, using ethylene glycol dimethacrylate as crosslinking agent. The hydrogels can be made pH-sensitive or temperature-sensitive, by incorporating methacrylic acid, or N-isopropylacrylamide, as monomers [Elizabeth FR, et.al; 2006].

Suspension polymerization

This method is employed to prepare spherical hydrogel microparticles with size range of 1 µm to 1mm. In suspension polymerization, the monomer solution is dispersed in the non-solvent forming fine droplets, which are stabilized by the addition of stabilizer. The polymerization is initiated by thermal decomposition of free radicals. The prepared microparticles then washed to remove unreacted monomers, crosslinking agent, and
initiator. Hydrogel microparticles of poly(vinyl alcohol) and poly(hydroxy ethyl methacrylate) have been prepared by this method [Elizabeth FR, et.al; 2006].

**Polymerization by irradiation**

High energy radiation like gamma and electron beam, have been used to prepare the hydrogels of unsaturated compounds. The irradiation of aqueous polymer solution results in the formation of radicals on the polymer chains. Also, radiolysis of water molecules results in the formation hydroxyl radicals, which also attack the polymer chains, resulting in the formation of macroradicals. Recombination of the macroradicals on different chains results in the formation of covalent bonds, and finally a crosslinked structure is formed. During radiation, polymerization macroradicals can interact with oxygen, and as a result, radiation is performed in an inert atmosphere using nitrogen or argon gas. Examples of polymers crosslinked by radiation method include poly (vinyl alcohol), poly (ethylene glycol), poly (acrylic acid). The major advantage over chemical initiation is the production of relatively pure, residue-free hydrogels [Park K, et.al; 1993].

**Chemically crosslinked hydrogels**

Polymers containing functional groups like -OH, -COOH, -NH$_2$ are soluble in water. The presence of these functional groups on the polymer chain, can be used to prepare hydrogels by forming covalent linkages between the polymer chains and complementary reactivity, such as amine-carboxylic acid, isocyanate-OH/NH$_2$ or by Schiff base formation. Gluteraldehyde can be used as a crosslinking agent to prepare hydrogels of polymers containing -OH groups like poly (vinyl alcohol). Also, polymers containing amine groups (albumin, gelatin, polysaccharides) can be crosslinked using gluteraldehyde.

Polymers that are water soluble can be converted to hydrogels, using bis or higher functional crosslinking agents like divinylsulfone, and 1, 6-hexanedi dibromide. The crosslinking agents react with the functional groups present on the polymer, via addition reactions. These crosslinking agents are highly toxic, and hence unreacted agents have to be extracted [Rosiak JM, et.al; 1995].

**Physically crosslinked hydrogels**

Most of the covalent crosslinking agents are known to be toxic, even in small traces. A method to overcome this problem and to avoid a purification step is to prepare hydrogels by reversible ionic crosslinking. Chitosan, a polycationic polymer can react with positively charged components, either ions or molecules, forming a network through
ionic bridges between the polymeric chains. Among anionic molecules, phosphate bearing groups, particularly sodium tripolyphosphate is widely studied. Ionic crosslinking is a simple and mild procedure. In contrast to covalent crosslinking, no auxiliary molecules such as catalysts are required. Chitosan is also known to form polyelectrolyte complex with poly (acrylic acid). The polyelectrolyte complex undergoes slow erosion, which gives a more biodegradable material than covalently crosslinked hydrogels [Rosiak JM, et.al; 1999].

1.4.6 Choice of method for preparation of hydrogel

Hydrogels can be obtained by radiation technique in a few ways, including irradiation of solid polymer, monomer (in bulk or in solution) or aqueous solution of polymer. The first method, i.e. irradiation of hydrophilic polymer in a dry form, has some drawbacks. It requires special sample preparation (like pressing or melting), and some difficulties are encountered in obtaining homogeneous macroscopic hydrogels. Moreover, it requires usually much higher doses of ionizing radiation to obtain a gel compared to irradiation in solution, and, furthermore, it is difficult to remove fully the oxygen, which promotes unwanted side reactions. One of the reasons for the high gelation doses in dry state is that radiation-chemical yield of radicals that are the precursors of crosslinks, is usually lower than in aqueous solution. Also the restricted motion of the radical-bearing chains limits the effectiveness of crosslinking. Radiation doses are calculated as energy per mass unit of the system. In this technique polymerization takes place in the first stage, followed by crosslinking of the formed chains [Nedkov ES, et.al; 1994].

Especially convenient method of radiation-based synthesis of hydrogels is the irradiation of polymers in aqueous solution, such systems, containing neither monomers nor crosslinking agents are easier to control and study. With the application of this method, lower number of usually unwanted processes occurs, as e.g. homografting of monomer on a polymer chain that may lead to branched structures, and hydrogels formed by this way are suitable for biomedical use with no need of further purification [Kabanov VY; 1998].

Typical examples of simple, synthetic polymers used for hydrogel formation by this method are poly(vinyl alcohol) - PVAL, polyvinylpyrrolidone - PVP, poly(ethylene oxide) - PEO, polyacrylamide - PAAm, poly(acrylic acid) - PAA and poly(vinyl methyl ether) - PVME. Gels obtained from two latter substrates belong to the group being of
particular interest as the components of intelligent biomaterials, since their properties are sensitive to environmental stimuli - pH, ionic strength (PAA) and temperature (PVME) [Peppas NA, et.al; 1976].

1.5 Hydrogels as Biomaterials

Polymeric hydrogels, owing to their dynamic structural properties have been commonly utilized in numerous biomedical and agricultural applications. The biomedical applications of hydrogels are classified into three distinct categories namely, coatings such as catheters, homogeneous materials such as contact lenses and devices such as sustained drug delivery systems [Mack EJ, et.al; 1987]. Though hydrogels are applicable in variety of areas, hydrogels as biomaterials is the area of concern for this research work.

Hydrogels resemble of living tissue in physical properties more than any other class of synthetic biomaterials. The high water content and the soft rubbery consistency of hydrogels contribute to their superficial resemblance of human tissues. This also contributes to their biocompatibility by minimizing mechanical irritation to surrounding tissue [Peppas NA; 1986]. The wide range of biomedical applications of hydrogels are attributed to both their satisfactory performance upon in vivo implantation in either blood contacting or tissue contacting situations and to their ability to be fabricated into a wide range of morphologies [Ratner BD; 1989].

The various applications of hydrogels in the biomedical field are summarized in table 1.3.

Table 1.3 Biomedical Applications of Synthetic Hydrogels

<table>
<thead>
<tr>
<th>Coatings</th>
<th>“Homogeneous” Materials</th>
<th>Devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sutures</td>
<td>Electrophoresis gels</td>
<td>Enzyme therapeutic systems</td>
</tr>
<tr>
<td>Catheters</td>
<td>Contact lenses</td>
<td>Artificial organs</td>
</tr>
<tr>
<td>IUD’s</td>
<td>Artificial corneas</td>
<td>Sustained drug delivery systems</td>
</tr>
<tr>
<td>Blood detoxicants</td>
<td>Vitreous-humour replacements</td>
<td></td>
</tr>
<tr>
<td>Sensors</td>
<td>Oestrous –Inducer</td>
<td></td>
</tr>
<tr>
<td>Vascular grafts</td>
<td>Soft tissue substitutes</td>
<td></td>
</tr>
<tr>
<td>Electrophoresis cells</td>
<td>Burn dressings</td>
<td></td>
</tr>
<tr>
<td>Cell structure substrates</td>
<td>Bone ingrowth sponges</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dentures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ear drum plugs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synthetic cartilages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemodialysis membranes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Particulate carriers of tumour antibodies</td>
<td></td>
</tr>
</tbody>
</table>
The area of interest presented in this work is that of application of hydrogels for wound dressings and controlled drug release devices for advanced wound management.

1.5.1 Topical application of hydrogels as wound dressings

Hydrogels are available in an amorphous form or as sheet dressings. Because of their high water content and presence of hydrophilic sites, hydrogels can absorb excess wound exudates while producing a moist wound environment. Hydrogels are used to debride a wound by rehydration and promotion of autolysis.

Hydrogels are used in a variety of wounds including pressure sores and cavity wounds. They are suitable for lightly exuding wounds, necrotic tissues, slough and shallow granulating wounds. Hydrogels also can ease the pain of radiotherapy burns and sooth as well as heal chapped or macerated skin [Nedkov ES, et.al; 1994]. Hydrogels should be applied directly onto a wound and secured with a secondary dressing. Hydrogels are the most preferred choice of wound dressing due to following properties [Bradley M; 1998].

- soft & elastic, but mechanically strong enough
- good adhesion to the wound without tendency for excessive sticking, therefore enable painless removal or exchange of dressings without disturbing healing process
- transparent so that healing process can be monitored
- enable easy treatment of wounds with drugs
- absorb exudates & bacterial toxins
- non-antigenic & do not provoke allergic reactions
- soothe pain & provide optimal wound healing

1.5.2 Drug Delivery Devices

Hydrogels are very versatile materials and have attracted significant attention recently as drug delivery systems. In addition their inertness and good compatibility, the ability of hydrogels to release an entrapped drug in an aqueous medium and the ease of regulating such drug release make hydrogels particularly suitable as drug carriers for the controlled release of pharmaceuticals.

In recent years major emphasis has been put on the study of polymeric hydrogels in biomedical research related to drug delivery due to their dynamic properties. Hydrogels have to be biocompatible and biodegradable to be ideal for drug delivery applications. The degradation products should be non-toxic and should not cause an inflammatory
response. The degradation should also occur within a reasonable period as required by the application [Park K, et.al; 1993].

**1.5.3 Mechanisms of Controlled Drug Delivery**

A convenient classification of controlled-release systems is based on the mechanism that triggers the release of the incorporated drug.

**Table 1.4 Classifications of Controlled Release Systems**

<table>
<thead>
<tr>
<th>Type</th>
<th>Controlling step</th>
<th>Drug release mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion-controlled devices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reservoir (membrane) devices</td>
<td>Concentration difference</td>
<td>Diffusion</td>
</tr>
<tr>
<td>Matrix (monolithic) devices</td>
<td>Concentration difference</td>
<td>Diffusion</td>
</tr>
<tr>
<td>Chemically-controlled devices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biodegradable (bioerodible) devices</td>
<td>Degradation</td>
<td>Reaction-dependent diffusion</td>
</tr>
<tr>
<td>Pendant chain devices</td>
<td>Hydrolysis</td>
<td>Reaction and Diffusion</td>
</tr>
<tr>
<td><strong>Solvent-activated systems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmotically controlled devices</td>
<td>Osmosis</td>
<td>Osmotic flow</td>
</tr>
<tr>
<td>Swellable systems</td>
<td>Swelling</td>
<td>Diffusion</td>
</tr>
<tr>
<td>Swelling-controlled systems</td>
<td>Swelling front</td>
<td>Relaxation-dependent Diffusion</td>
</tr>
<tr>
<td><strong>External force-induced release</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetically controlled devices</td>
<td>Magnetic field</td>
<td>Diffusion</td>
</tr>
</tbody>
</table>

**1.5.4 Drug release mechanisms from hydrogel devices**

Hydrogels have a unique combination of characteristics that make them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water. Therefore, the molecule release mechanisms from hydrogels are very different from hydrophobic polymers. Mechanisms for controlled release of drug from hydrogels are characterized as follows:

1. Diffusion-controlled
2. Swelling-controlled
3. Chemically-controlled

**1.5.4.1 Diffusion-Controlled Release**

Diffusion-controlled release is the most widely applicable mechanism for describing drug release from hydrogels. Fick's law of diffusion with either constant or variable diffusion coefficients is used in modeling diffusion-controlled release. In diffusion systems the therapeutic drug, which may be either encapsulated in the polymer membrane or suspended within the polymer matrix passes through the polymer that forms the controlled release device when placed in an aqueous media [Peppas NA, et. al;
The medium diffuses into the matrix, dissolves the incorporated drug, which then diffuses out of its carrier. The diffusion can occur on a macroscopic scale as through pores in the polymer matrix, or at molecular level by passing between chains as that of reservoir devices. In the matrix system the rate of release is time dependent.

In reservoir devices the active ingredient within the polymer matrix forms a core surrounded by an inert polymeric film or membrane, which acts as the diffusion barrier. This membrane surrounding the reservoir is the only structure, which effectively limits the release of the drug molecule in such systems.

1.5.4.2 Swelling-Controlled Release Systems

Swelling-controlled release occurs when diffusion of drug is faster than hydrogel swelling. The modeling of this mechanism involves moving boundary conditions where molecules are released at the interface of rubbery and glassy phases of swollen hydrogels.

The release of the drug could occur from matrix or reservoir devices. The coupling of diffusion and the macromolecular relaxation of the carrier control the release mechanism of the incorporated drug providing conditions for zero-order release. A typical polymeric swelling-controlled release system is depicted in Figure 1.17. The dry polymer slab when placed in an aqueous medium swells thus increasing its aqueous solvent content within the formulation as well as the polymer mesh size as a result, allowing the incorporated drug to diffuse out into the host environment [Peppas NA, et.al; 1993].
Solute Transport in Swelling-Controlled Release Systems

The incorporated drug is essentially immobile in the glassy region of the polymer but begins to diffuse out as the polymer swells in the compatible penetrant medium. The release of drug thus depends on two simultaneous rates processes, medium migration into the polymer network and the solute diffusion out of the network. The solubility of the drug for a given medium is also essential.

In swelling-controlled release systems, the polymer has to swell to some extent before the drug can diffuse out, thus the initial burst effect of the drug is observed. The continued swelling of the polymer eases the diffusion of the drug, ameliorating the slow tailing off of the release curve. The net effect of the swelling process is to prolong and linearize the release profile of the drug [Chien CL, et.al; 2003]. A schematic representation of the swelling-controlled release action is illustrated in Figure 1.18.

Figure 1.17 Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems

Figure 1.18 Schematic representation of a swelling controlled release system

The penetrant medium (M) enters the initially glassy polymer (P) with velocity (υ). The incorporated (D) drug diffuses through the swollen gel layer (G).
**Fick’s Laws of Diffusion**

Understanding the mechanisms of the penetrant medium diffusion into the swellable polymer is crucial to define the release profile of the incorporated solute. Fick’s first law is described by Equation 1 where $j$ is the flux per unit area, $A$ is the area across the diffusional field, $D$ is the diffusional coefficient, $c$ is the concentration of solute, $z$ is the distance and $\partial c / \partial z$ is the concentration gradient across the $z$ axis.

$$j = -A \frac{\partial c}{\partial z}$$

*Equation 1*

The law states that the flux of a component of concentration across a membrane of unit area, in a predefined plane, is proportional to the concentration differential across that plane.

Fick’s second law with constant boundary conditions can successfully describe much of the observed solute transport through polymers. It is successful in describing transport both above and below $T_g$. It states that the rate of change of concentration in a volume element of a membrane, within the diffusional field, is proportional to the rate of change of concentration gradient at that point in the field.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

The boundary conditions are:

$t = 0$ \hspace{1cm} $-1/2 < x < 1/2$ \hspace{1cm} $C = C_I$

$t > 0$ \hspace{1cm} $x = \pm 1/2$ \hspace{1cm} $C = C_0$

*Equation 2*

**Fickian and Non-Fickian Diffusion**

Diffusion through polymers is associated with the physical properties of the gel network and the interaction between the polymer and the penetrant medium. Based on Fick’s law of diffusion, diffusional behaviours are classified as Fickian (Case I) and non-Fickian (Case II and anomalous) in swellable polymers. The propositions were made in accordance to the penetrant diffusion rate and the polymer relaxation rate.

In the Fickian (Case I) diffusion the penetrant mobility rate is much lower than the segmental relaxation rate. The reduced driving concentration gradient slows down the diffusion rate in the polymer slab geometry. In non-Fickian (Case II) diffusion, however, the mobility rate of the penetrant is much higher than the segmental relaxation rate. The
sharp boundary between the gel phase formed by the penetrant and the glassy portion of the polymer becomes the rate-determining step. Anomalous diffusion behaviour is characterized by the intermediate properties between the Fickian Case I and Case II behaviour [Peppas NA, et.al; 1993].

Time dependent swelling behaviour in swellable polymers has been generally described in the literature according to Equation 3, termed as the power-law model, with $n$ being the diffusional exponent.

$$\frac{M_t}{M_\infty} = Kt^n$$

*Equation 3*

$M_t/M_\infty$ represents the fractional uptake of the penetrant medium or release of the incorporated solute at time $(t)$ normalized with respect to equilibrium conditions. The $k$ value is a constant, which incorporates the characteristics of the macromolecular network/drug system and the dissolution medium.

The parameter $n$ determines the dependence of the medium uptake or release rate on time.

Table 1.5 summarizes a list of possible transport mechanisms with their characteristic $n$ values and time dependence.

**Table 1.5 Transport mechanisms of penetrant through a polymer slab**

<table>
<thead>
<tr>
<th>Exponent $n$</th>
<th>Type of transport</th>
<th>Time dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Fickian diffusion</td>
<td>$f(t^{-0.5})$</td>
</tr>
<tr>
<td>$0.5 &lt; n &lt; 1.0$</td>
<td>Non-Fickian diffusion (anomalous)</td>
<td>$f(t^{-n\frac{1}{2}})$</td>
</tr>
<tr>
<td>1.0</td>
<td>Case II transport</td>
<td>Time-independent</td>
</tr>
<tr>
<td>$n &gt; 1.0$</td>
<td>Super Case II transport</td>
<td>$f(t^{-n\frac{1}{3}})$</td>
</tr>
</tbody>
</table>

**Influential Factors in Swelling-Controlled Release Systems**

One of the most useful features of a polymer's swelling ability manifests itself when the swelling can be triggered by a change in the environment surrounding the delivery system. The external environmental conditions involve pH, temperature, magnetic field or ionic strength. The gels either shrink or swell in response to such environmental changes as illustrated in Figure 1.19.

The effect of the environmental conditions on the polymer’s performance is dependent on the nature of the polymer, which could be ionic or neutral. The swelling release action
in neutral hydrogels is driven by the thermodynamic mixing contribution of the penetrant medium and the polymer to the overall free energy, which is coupled with an elastic polymer contribution. In ionic hydrogels the driving forces are the same as that of neutral gels along with some additional contributors such as the ionic interactions between the charged polymer and the free ions. For most of the polymers, the structural changes are reversible and repeatable upon additional changes in the external environment. Hydrogels can be synthesized appropriately to achieve the desired response from a given environmental condition. Parameters such as the polymer composition, degree of crosslinking density and the size and nature of the incorporated drug molecule play an important role in determining the drug release behaviour and thus must be considered during the design of swelling-controlled release devices [Chien CL, et.al; 2006].

Effect of the Polymer Composition
The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics. Presence of hydrophilic components in the polymer network enhances the swelling characteristics of the polymer. Hydrophobic components on the other hand reduce the swelling efficiency.

Effect of the Crosslinking Density
Increase in crosslinking density through addition of crosslinking agents such as divinyl glycol (DVG), divinyl benzene (DVB) or tripropyleneglycol diacrylate (TPGDA) are known to reduce the equilibrium swelling. Reduced swelling is marked with reduced diffusion coefficient.

Effect of the Environmental pH
Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendant functional groups. The equilibrium swelling behaviour of ionic hydrogels containing acidic and/or basic functional groups is illustrated in Figure 1.20.

Peppas studied the swelling behaviour of pH sensitive anionic hydrogels based on HEMA, methacrylic acid and maleic anhydride in varied pH environments. They reported low swelling activity of the hydrogels in acidic medium but very high swelling activity in basic medium.

![Equilibrium degree of swelling in response to pH](image)

**Figure 1.20** Equilibrium degree of swelling in response to pH

Most useful pH-sensitive polymers swell at high pH values and collapse at low pH values, the triggered drug delivery occurs upon an increase in the pH of the environment. Such materials are ideal for systems such as oral delivery, in which the drug is not released at low pH values in the stomach but rather at high pH values in the upper small intestine [Chien CL, et.al; 2006].

**Effect of the Environmental Temperature**

Changes in the environmental temperature either enhance the swelling ability of the hydrogel or in contrary could cause the hydrogel to collapse. Physical gels that contain hydrophilic components exhibit enhanced swelling behaviour at elevated temperatures and are referred to as thermo-swelling gel. However, gel networks composed of relatively hydrophobic components shrink at elevated temperatures. These networks are referred to as thermoshrinking networks. Thermoshrinking gels undergo reversible swelling and de-swelling in response to changes in environmental temperature [Park K, et.al; 1993].
Effect of the Ionic Strength

According to the concept of Donnan equilibrium, an increase in the ionic strength of the swelling agent increases the ionization of a weakly polyelectrolyte system thus leading to high swelling activity. However, once the ionic hydrogel has been fully ionized, further increase in the ionic content of the swelling agent will cause the hydrogel to de-swell due to the screening effect of the counterions. Anionic gels are normally unionized at a pH lower than the gel pKa while cationic gels display the opposite behaviour and the pH is dependent on the pKb of the gel.

Khare and Peppas in their study on anionic hydrogels observed a decrease in the swelling activity upon a further increase in the ionic strength of the swelling agent at a constant pH higher than the pKa of the gel.

Effect of the Nature and Size of the Drug

The size and the nature of the incorporated drug also play an important role in determining the efficiency of its release from the carrier. An increase in the molecular size of the drug reduces the drug release rate [Rosiak JM, et.al; 1995].

Based on all above description of synthesis and properties of hydrogels following criteria is designed for development of hydrogels for drug delivery. Since materials selection and network fabrication governs the rate and mode of drug release from hydrogel matrices. Several design criteria are crucial for drug delivery formulations and have to be evaluated prior to hydrogel fabrication and drug loading [Gen S; 1989, Gudeman LF, et.al; 1995, Bradley M, et.al; 1998].

Poly vinyl alcohol is a well known biologically friendly polymer and has been developed for biomedical applications such as wound dressing, artificial skin, and cardiac device etc. So PVA is used as a polymer for development of hydrogel for wound dressing application.
**Introduction**

**Research Insight**

Discovery and introduction of Absorbable gelatin sponges and Polymeric hydrogels, into the wound management in the early 1960s have been of great research interest. Numerous researchers around the globe have carried out extensive research on absorbable haemostats and hydrogels and this has resulted in some very classical and important developments in such materials. Despite a great number of papers and patents devoted towards development of advanced wound management products, there is only limited number of successfully commercialized technologies of production of drug loaded haemostats and hydrogel biomaterials.

Absorbable gelatin sponges are biodegradable haemostats which are used to prevent bleeding during surgical procedures and are biodegradable without producing any adverse effects. Surgifoam by Johnson and Johnson is the commercially successful example of the absorbable gelatin sponge while drug loaded absorbable gelatin sponges are still under investigation [Israel N, et.al; Patent WO/2009/109963; 2009].

The hydrogel materials for wound healing are used in direct contact with living tissues. They prevent contamination of a wound by microorganisms from outside, inhibit the loss of body fluids, deliver oxygen to the wound, and accelerate healing processes. A commercially successful example of such a dressing is the hydrogel dressing known under the trade name Aquagel and marketed mainly in the Central Europe. However, drug loaded hydrogels are still under research [Anthony B, et.al; Patent WO/2009/132153; 2009].

There still seems to be an infinite range of possible applications of such versatile materials for wound management. Thus there remains an everlasting quest to achieve superiority over present wound management systems in terms of biocompatibility, mechanical strength, response to environment and economy to meet the requirements of such applications.

The need for cheaper, more responsive and more biocompatible substitutes drug delivery systems for wound dressings prompted this research project. Research work presented here is focused on development of absorbable haemostats and hydrogels and their drug loading for advanced wound management.
Research Direction
The direction of the research in this Ph.D. project is geared towards obtaining absorbable gelatin sponges and hydrogels for wound management as well as for controlled drug delivery applications through an economical and efficient polymerization process. The wound management systems would possess enhanced properties such as mechanical strength, controlled drug release properties and biocompatibility. Absorbable gelatin sponges will be prepared by using natural polymers and will be further loaded with antiseptic and anesthetic drugs which will contribute to advanced wound management. Hydrogels will be prepared from a range of polymers which will contribute to wound healing.

In order to proceed with the polymerization process, chemical crosslinking will be tried but residual chemicals remaining after crosslinking may be a major issue towards safety of use of hydrogels for wound healing. The residual chemical crosslinking agents may leach out from the hydrogel while application and can create toxicity to the object. Polymerization using UV radiations normally requires the presence of a photoinitiator in the reacting monomer mixture. A photoinitiator is a photosensitive chemical, which is converted into reactive radicals upon exposure to the UV light. Photoinitiators besides being costly if not completely utilized in the polymerization process can lead to undesirable toxic impurities trapped in the polymer matrix that may leach out of the matrix in a biomedical application. This has been a major issue pointed by a number of research publications.

To avoid these issues, gamma radiation will be used for preparation of hydrogels for wound healing. This preferred method is nontoxic, initiator free and also helps in sterilization of materials along with crosslinking by radiation.

The research outcomes of this project will help in design and development of surgical dressings for advanced wound management as next generation wound care systems. The research work was planned as per the plan of work given in section 1.6. Wound infection and Pain are the chief complaints of patients suffering from wound injury. For the long time, gauze soaked with antibiotic and anesthetic drug is used for treatment of such wounds. In this study, Gentamicin sulphate was selected as the model drug with antibacterial activity and lidocaine hydrochloride with anesthetic activity. Attempt was made to polymerize PVA and PVA along with drug using chemical polymerization and gamma polymerization techniques.
Plan of work

Drug candidates tried for the research work:

i. Gentamicin sulphate
ii. Lidocaine Hydrochloride

1. Preformulation studies

I. Standardization of Drugs and Excipients
II. Drug-Excipient compatibility studies
III. Analytical Method Development

2. Formulation and evaluation of drug loaded gelatin sponges

A] Formulation and evaluation of absorbable gelatin sponges
   I. Formulation Development
   II. Characterization and evaluation
   III. Scale up and reproducibility studies

B] Formulation and evaluation of drug loaded absorbable gelatin sponges
   I. Formulation Development of biodegradable microspheres.
   II. Characterization and evaluation of microspheres
   III. Incorporation of drug loaded microspheres into sponge
   IV. Characterization and evaluation of drug loaded gelatin sponges

3. Formulation and evaluation of drug loaded hydrogels

A] Formulation and evaluation of hydrogels
   I. Formulation Development of hydrogels
   II. Characterization and evaluation
   III. Scale up and reproducibility studies

B] Formulation and evaluation of drug loaded absorbable gelatin sponges
   I. Incorporation of drug into hydrogels
   II. Characterization and evaluation of drug loaded hydrogels

4. Stability studies of developed formulations

A] Stability studies of existing formulations
   I. Absorbable gelatin sponges
   II. Hydrogels

B] Stability studies of new formulations
I. Drug loaded absorbable gelatin sponges
II. Drug loaded hydrogels

5. Preclinical evaluation of developed formulations
   A) Absorbable gelatin sponges
      I. Skin irritation testing
      II. In vivo biodegradation
      III. Incision wound healing efficacy
   B) Hydrogels
      I. Skin irritation testing
      II. Excision wound healing efficacy
   C) Drug loaded absorbable gelatin sponges
      I. Skin irritation testing
   D) Drug loaded Hydrogels
      I. Skin irritation testing

The work proposed in this plan of work has been presented in following chapters.