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Development and Characterization of Heparin Injection: A Comprehensive Review

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ABSTRACT

This article presents a comprehensive pharmaceutical and clinical overview of Heparin, a widely utilized parenteral anticoagulant that remains a cornerstone in the management and prevention of thromboembolic disorders. Structured through a module-based approach, the article examines heparin across four interconnected domains. The first module discusses the rationale for selecting heparin as a representative agent within the anticoagulant class. The second module focuses on preformulation studies, including physicochemical characterization, which is essential for injectable development. The third module explores formulation and manufacturing processes, highlighting sterile production techniques and addressing key challenges in parenteral formulation. The final module involves laboratory-based evaluation and quality control testing of the developed heparin injection, alongside a discussion of applicable regulatory frameworks and marketing strategies. This article aims to integrate theoretical knowledge with practical experience, offering a multidisciplinary understanding of heparin's role in pharmaceutical development and clinical therapeutics.

Keywords : Heparin, formulation, sterility test, labelling

INTRODUCTION

Heparin is the earliest and most widely used anticoagulant and antithrombotic drug that is still used in a variety of clinical indications. Since it was discovered in 1916, after more than a century of repeated exploration, heparin has not been replaced by other drugs, but a great progress has been made in its basic research and clinical application. Besides anticoagulant and antithrombotic effects, heparin also has antitumor, anti-inflammatory, antiviral, and other pharmacological activities. It is widely used clinically in cardiovascular and cerebrovascular diseases, lung diseases, kidney diseases, cancer, etc., as the first anticoagulant medicine in COVID-19 exerts anticoagulant, anti-inflammatory and antiviral effects. At the same time, however, it also leads to a lot of adverse reactions, such as bleeding, thrombocytopenia, elevated transaminase, allergic reactions, and others.[2] [1]

MECHANISM OF ACTION

Once administered, heparin binds to several proteins; however, it is binding to an antithrombin that is important, as this causes a surface change and inactivates thrombin. Binding to antithrombin blocks several different factors of the clotting cascade, but two are predominant: thrombin

(Factor IIa and Factor Xa). By inactivating thrombin, it blocks the conversion of fibrinogen to fibrin;this prevents the formation of clots and prolongs the clotting time of blood. Heparin does not affect bleeding time, but it does prolong the time that blood takes to clot.[3][4]

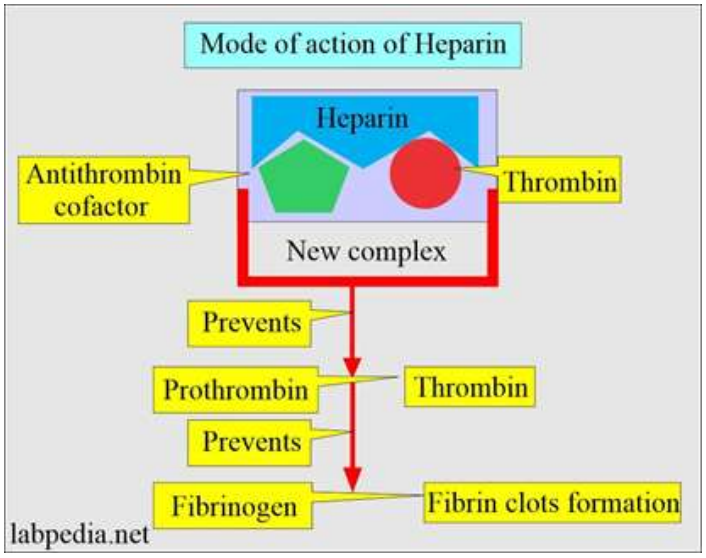


Fig no.1: mechanism of Heparin

ABSORPTION

Heparin is not absorbed through the gastrointestinal tract and is therefore administered via a parenteral route. Peak plasma concentration and the onset of action are achieved immediately after intravenous administration.

VOLUME OF DISTRIBUTION

Tissues, clinicians should use actual body weight in obese patients to account for extra vasculature. The volume of distribution is 0.07 L/kg. Although heparin does not distribute into adipose.

PROTEIN BINDING

Heparin is highly bound to antithrombin, fibrinogens, globulins, serum proteases, and lipoproteins.

METABOLISM

Heparin does not undergo enzymatic degradation.

ROUTE OF ELIMINATION

Heparin undergoes biphasic clearance, a) rapid saturable clearance (zero-order process due to binding to proteins, endothelial cells, and macrophages), and b) slower first-order elimination. Low doses of heparin are cleared mostly by a saturable, rapid, zero-order process. Slower firstorder elimination usually occurs with very high doses of heparin and is dependent on renal function. High-molecular-weight moieties are cleared more rapidly than lower molecular-weight

moieties.[15]

ADVERSE EFFECTS

- Easy bleeding and bruising.
- Pain, redness, warmth, irritation, or skin changes where the medicine was injected.
- Itching of your feet.
- Bluish-coloured skin.
- Skin warmth or discoloration.
- Chest pain.

FORMULATIONS

1. Injectable Forms (Most Common):

a. Intravenous (IV) Injection/Infusion

- Form: Solution for injection.
- Concentration: Varies (e.g., 1,000 units/mL, 5,000 units/mL, 10,000 units/mL).

b. Subcutaneous (SC) Injection

- Form: Prefilled syringes or multi-dose vials.
- Use: Prophylaxis or treatment of thromboembolic disorders, especially in outpatient settings.

2. Prefilled Syringes:
   - Contain a fixed dose of heparin or LMWH (like enoxaparin).
   - Available in different strengths (e.g., 40 mg, 60 mg, 100 mg enoxaparin).
3. Topical Form (for Heparin Sodium):
   - Form: Gel or ointment.
   - Brand Examples: Heparinoid cream, Hirudoid, etc.
   - Use: Relief of pain and inflammation in superficial thrombophlebitis, bruises, or soft tissue injuries.

Fig no.2: Injectable form of Heparin

PREFORMULATION STUDIES

1. ORGANOLEPTIC CHARACTERS:

A typical preformulation program should begin with the description of the drug substance. Its color is colorless to straw colored, odourless and clear solution.[14]

2. MELTING POINT

In preformulation, it is a critical physicochemical property that provides insight into a drug's purity, thermal stability, and polymorphism.

Melting point of Heparin is >181°C.

3. DISSOCIATION CONSTANT (pKa):

The dissociation constant is a value that describes the extent to which a compound ionizes or dissociates into its ions when dissolved in water. Many drugs are either weakly acidic or basic compounds and, in solution, depending on the pH value, exist as ionized or un-ionized species.

The relative concentrations of un-ionized and ionized forms of a weakly acidic or basic drug in a solution at a given pH can be readily calculated using the Henderson-Hasselbalch equations:

For bases: $pH = pKa + \log \frac{\text{unionized}}{\text{ionized}}$

For acids: $pH = pKa + \log \frac{\text{ionized}}{\text{unionized}}$

 pKa value of Heparin is approximately 5.1 and its pKa value reflects the acidity of Heparin.

4. PARTITION COEFFICIENT:

The partition coefficient, denoted as log P, is a common measure used to characterize the lipophilicity of an organic molecule and is defined as the equilibrium of the distribution of the non-ionized compound concentration between the organic and aqueous phases.

$$\text{Log P} = \log \frac{\text{unionized compound(org)}}{\text{unionized compound(aqs)}}$$

The relative lipid solubility of a pharmacological ingredient can be deduced by observing how it disperses between water and an immiscible organic solvent. [6]

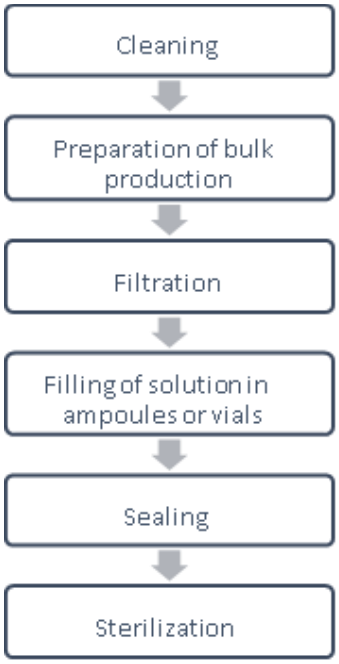
 Log P of Heparin is -2.33.

5. HYGROSCOPICITY:

Many drug substances exhibit a tendency to absorb moisture. The amount of moisture adsorbed by a fixed weight of anhydrous sample in equilibrium with the moisture of the air at a given temperature. These are classified as Deliquescent, and Hygroscopic. This process depends on the relative humidity of the surroundings. [5] Heparin is moderately hygroscopic in nature.

FORMULATION OF HEPARIN INJECTION

Steps involved in production of parenteral are:



 Step 1: Equipment cleaning

- Clean all stainless-steel mixing tanks, pipelines and filters with validated CIP/SIP procedures.
- Sterilize filling equipment, tanks and filters via steam-in-place (sip) or autoclaving.

 Step 2: Bulk Solution preparation

Formulation of Heparin Sodium Injection (5,000 IU/mL):

Table no.3: Ingredients of Heparin injection

a. Fill mixing tank with 50-60% of required volume of water for injection (WFI).
b. Dissolve sodium chloride with continuous mixing.
c. Add Heparin sodium slowly with gentle mixing (prevent frothing or mechanical shearing).
d. Add remaining WFI to bring up to final volume.
e. Check and adjust pH to about 7.0-8.0 using dilute HCl or NaOH under nitrogen purge.

➢ **Step 3: Filtration**

- Sterile filtration through a 0.22 µm membrane filter.
- Use double filtration for safety if required.
- Collect filtrate in a sterile receiving tank.

➢ **Step 4: Aseptic filling**

- Transfer filtered solution to the aseptic filling zone.
- Use peristaltic or piston pumps to fill sterile vials or ampoules.

➢ **Step 5: Sealing**

- Immediately seal vials with sterile rubber stoppers and aluminium caps.
- Ampoules are flame-sealed.

➢ **Step 6: Sterilization**

- Method: Sterile Filtration (0.22-micron filter).
- Reason: Heparin is heat-sensitive and cannot be autoclaved.
- Procedure: Filter the heparin solution aseptically at room temperature (20–25°C) to ensure sterility without degrading the drug.
- Environment: Perform in an ISO Class 5 clean room to maintain sterility.

EVALUATION OF HEPARIN INJECTION

1. STERILITY TEST:

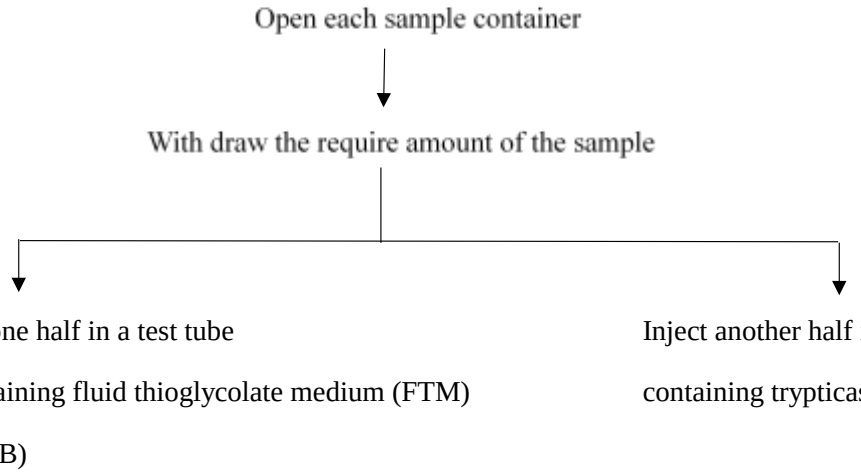
It is a method carried out to detect confirm absence of any viable form of microbes in product. The method used for sterility test are

a. Direct transfer method

b. Membrane filtration method

a) **Direct transfer method:**

It is a traditional method.



- Volume of the medium must be sufficient to promote and expedite microbial growth. Adequate mixing between the sample inoculum and the culture medium must take place to maximize interaction and facilitate microbial growth.

b) **Membrane filtration method (MF):**

The steps involved in MF sterility test method are:

1. The filter unit must be properly assembled and sterilized prior to use.
2. The contents are transferred to the filter assembly under strict aseptic conditions.
3. The membrane is removed aseptically.

4. Membrane is cut in half.
5. One half is place in suitable volume of FTM and another in an equal volume of TSB.

Interpretation of results:

1. If there is no visible evidence of microbial growth, it may be interpreted that the sample is without intrinsic contamination.
2. If microbial growth is found the sterility test may be repeated.

2. CLARITY TEST (PARTICULATE MATTER EVALUATION):

- Particulate matter in parenteral solutions has been recognized as an acceptable. Since the user could be expected to conclude that the presence of visible dirt would suggest that the product is of inferior quality.
- The entire product should be inspected by human inspectors under good light baffled against reflection into the eye and against black and white background.
- Any container with visible particle if seen is discarded. [12]

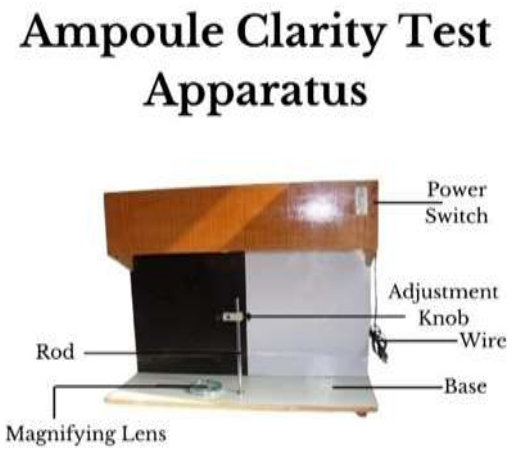


Fig no. 3: Clarity test apparatus

3.LEAKERS TEST: [CONTAINERS OR CLOSURES INTEGRITY TEST]

- Ampules sealed by fusion are subjected for leakers test.
- It is used to determine if any passage way remains to the outside, that may cause leakage of the contents or contamination.
- This test is usually performed by producing a negative pressure within an incompletely sealed ampule.

The ampule is entirely submerged in a deeply colored dye solution [1%methylene solution] After carefully rinsing the dye solution from the outside color from the dye will be visible with in a leaker. [11]

Fig no. 4: Methylene blue dye test

4.PYROGEN TEST:

- Presence of pyrogen may cause fever and alteration in blood coagulation.
- The tests used for pyrogen detection are:

a) Rabbit test b) LAL test

a) **Rabbit test: [Sham test]**

- Rabbits are used as the test animal because they show a physiological response to pyrogens, similar to that of human beings.
- Three healthy adult rabbits of either male or female, each weighing not less than 1.5kg are selected

Method:

Normal temperature is recorded prior to the test

↓

Dilute the test substance in pyrogen free saline test solution

↓

Warm the solution to 38.50 C

↓

Volume of injection is maintained between 0.5 to 10ml/kg

↓

Test solution is injected through an ear vein

↓

Body temperature is recorded by a clinical rectal thermometer

↓

Record temperature at an interval of 30mins for 3 hours

The difference between initial and final temperature is recorded. The difference in temperature should not be more than 1ºC.

Interpretation of Result:

1. The test is carried out on the first group of 3 rabbits; if necessary, on further groups of 3 rabbits to a total of 4 groups, depending on the results obtained.
2. Intervals of passing or failing of products are on the basis of summed temperature response. If the difference is negative, the result is counted as zero response[13].

NO. OF RABBITS	**INDIVIDUAL TEMP RISE (ºC)**	**TEMP. RISE IN GROUP (ºC)**	**TEST**
3 rabbits	0.6	1.4	Passes
(if above not passes): 3+5=8 rabbits	0.6	3.7	Passes

Table no.1: Interpretation result of Rabbit test

If above test not passes, the sample is said to pyrogenic.

Fig no.5: Rabbit test

b) LAL test:

- It is also known as limulus amoebocyte lysate [LAL test] or bacterial endotoxins test.
- The test is used to detect or quantify endotoxins of gram-negative bacterial origin.
- LAL test is based on the primitive blood clotting mechanism of the horse shoe crab.
- The presence of pyrogen is indicated by the formation of a proteinaceous gel, upon incubation of the mixture of LAL reagent and test solution.

Mechanism of LAL test:

Primitive blood clotting mechanism of horse shoe crab

↓

Enzymes located with the crabs amoebocyte blood cells endotoxins

↓

Initiation of an enzymatic coagulation cascade

↓

Proteinaceous gel

Pass-Fail test:

1. If a firm gel is formed -pyrogen present.
2. If an intact gel is not formed -pyrogen absent.[8]

PACKAGING OF HEPARIN INJECTION

1. Primary Packaging (Direct Contact)

Heparin Container Type	**Material**	**Volume Range**
Glass vials	Type I borosilicate glass	1 ml – 30 ml
Plastic vials/syringes	Medical-grade plastic (e.g., polypropylene)	1 ml– 10 ml
Prefilled syringes	Plastic or glass	1 ml – 5 ml
Ampoules (less common)	Glass (Type I)	1 ml – 5 ml

Table no.2: Primary packaging of Heparin containers

2. Secondary Packaging (Outer Packaging)

- Cartons/boxes made from pharmaceutical-grade cardboard.
- Used to hold individual or multiple units (e.g., 10 vials per box).
- Includes package insert/leaflet with usage instructions and warnings.

LABELLING OF HEPARIN INJECTION

LABEL ELEMENT	**DETAILS**
Brand name (if applicable)	E.g., "Heparin Sodium Injection"
Generic name	Heparin Sodium Injection, USP
Concentration	e.g., 1,000 USP units/mL or 5,000 USP units/mL
Volume	e.g., 1 mL, 5 mL
Total units per container	e.g., 5,000 units in 1 mL
Dosage form	Solution for injection
Route of administration	e.g., Intravenous (IV) or Subcutaneous (SC) use
Preservative status	"Preservative-free" or list of preservatives
Sterility statement	"Sterile"
Lot number	For traceability
Expiry date	As per stability studies
Storage instructions	"Store at 2–8°C. Do not freeze."
Manufacturer details	Name, address, license number

Table no.3: Labelling requirements of Heparin injection

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