IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, December 2025
Impact Factor: 7.67

Synthesis and New HPLC Validation Method of Low Molecular Weight Heparin

RNL Sirisha Adiraju, K. Bharathi, G. Lakshmi Jhansi, K. Rajini
Asst. Professor in Chemistry
Sir C. R. Reddy College of Engineering, Eluru

Abstract: *Low weight heparins (LMWH) have remained the most favorable form of heparin in clinics since 1990s' owing to its predictable pharmacokinetic properties. However, LMWH is mainly eliminated through kidney, thus limits its use in renal-impaired patients. In addition, the anticoagulant activity of LMWH is only partially neutralized by protamine. LMWH is obtained from a full-length, highly sulfated polysaccharide harvested from porcine mucosal tissue. The depolymerization involved in LMWH production generates a broad size distribution of LMWH fragments (6-22 sugar residues). This, combined with the various methods used to produce commercial LMWHs, result in variable pharmacological and pharmacokinetic properties. An alternative, chemoenzymatic approach offers a method for the synthesis of LMWH that has the potential to overcome the limitations of current LMWHs. This review summarizes the application of a chemoenzymatic approach to generate LMWH and the rationale for development of a synthetic LMWH.*

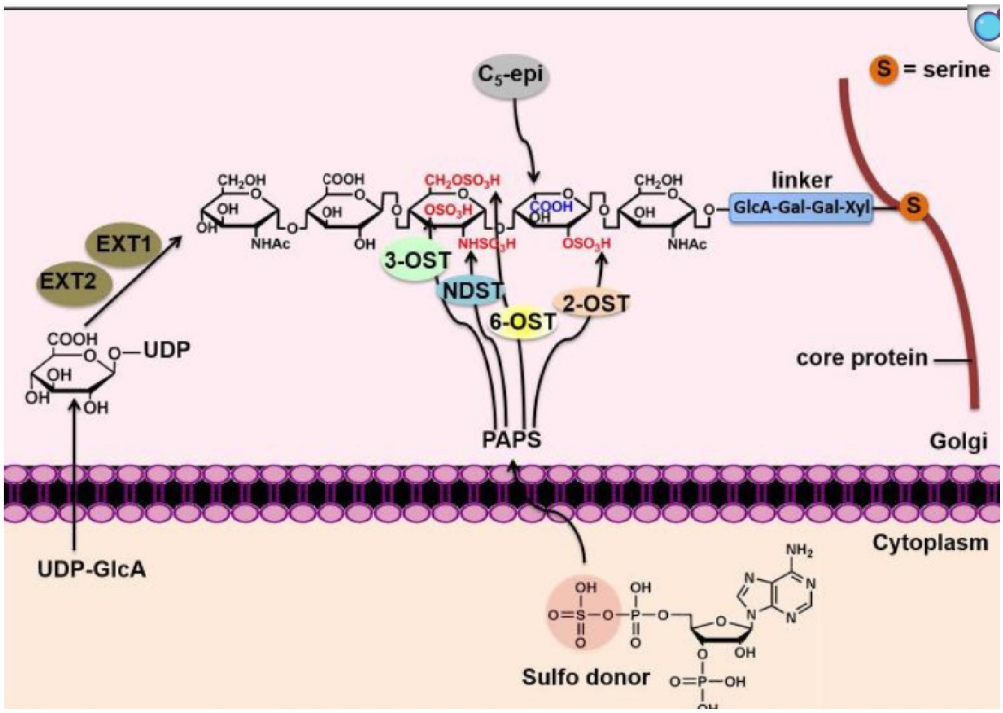
Keywords: Anticoagulants, Chemistry Techniques, Synthetic, Enzymes, Heparin, Low-Molecular-Weight, Protamines

I. INTRODUCTION

Biosynthesis of low molecular weight Heparin:

Biosynthesis of heparin occurs in the endoplasmic reticulum and the Golgi and involves a series of specialized enzymes. Generally, the pathway consists of three phases: initiation, polymerization and modification of the chain. The initiation phase involves formation of a linker that tethers to a serine residue of serglycin, a core protein presented in the mast cell .Then, chain polymerization takes place by formation of a copolymer of N-acetylglucosamine (GlcNAc) and GlcA, and this forms the backbone of heparin. This chain elongation process is driven by two polymerases known as exostosin glycosyltransferase 1 (EXT 1) and EXT 2 [56]. The unmodified polysaccharide backbone subsequently undergoes chain modification by a series of sulfations and epimerization. The first modification step is N-sulfation of GlcNAc residues by N-deacetylase/N-sulfotransferase (NDST), which is a critical step for subsequent modification. NDST has two functions: removal of the acetyl group (N-deacetylase activity, NDase) and installation of an N-sulfo group (N-sulfotransferase activity, NST) [57]. After N-sulfation, C5-epimerase (C5-epi) following by 2-O-sulfation take place. C5-epi converts the D-glucuronic acid (GlcA) to L-iduronic acid (IdoA) [58]. 2-O-sulfotransferase catalyzes the transfer of a sulfo group to the 2-O-position of either IdoA or GlcA, but preferentially to the IdoA residue [59]. The addition of a sulfo group at the 6-OH of GlcN is modified by 6-O-sulfotransferases [60]. Lastly, 3-O-sulfotransferases add a sulfo group at the 3-OH of GlcN (39), and this is critical for AT binding and anticoagulant activity [61, 62].

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, December 2025
Impact Factor: 7.67



HPLC validation method of Heparin sodium injection:

Heparin, is a medication and naturally occurring glycosaminoglycan. As a medication it is used as an anticoagulant (blood thinner). Specifically it is also used in the treatment of heart attacks and unstable angina. It is given by injection into a vein or under the skin. Other uses include inside test tubes and kidney dialysis machines.

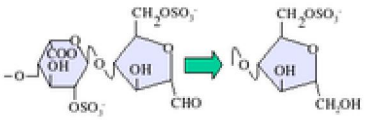
Common side effects include bleeding, pain at the injection site, and low blood platelets. Serious side effects include heparin-induced thrombocytopenia. Greater care is needed in those with poor kidney function.

Heparin is contraindicated for suspected cases of vaccine-induced pro-thrombotic immune thrombocytopenia (VIPIT) secondary to SARS-CoV-2 vaccination, as heparin may further increase the risk of bleeding in an anti-PF4/heparin complex autoimmune manner, in favor of alternative anticoagulant medications (such as argatroban or danaparoid).

Heparin appears to be relatively safe for use during pregnancy and breastfeeding

Native heparin is a polymer with a molecular weight ranging from 3 to 30 kDa, although the average molecular weight of most commercial heparin preparations is in the range of 12 to 15 kDa.Heparin is a member of the glycosaminoglycan family of carbohydrates (which includes the closely related molecule heparan sulfate) and consists of a variably sulfated repeating disaccharide unit. Either chemical or enzymatic depolymerization techniques or a combination of the two underlie the vast majority of analyses carried out on the structure and function of heparin and heparan sulfate.

Nitrous acid can be used to chemically depolymerize heparin/HS. Nitrous acid can be used at pH 1.5 or at a higher pH of 4. Under both conditions, nitrous acid effects deaminative cleavage of the chain.



IdoA(2S)-aMan: The anhydromannose can be reduced to an anhydromannitol

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 4, December 2025 Impact Factor: 7.67

At both 'high' (4) and 'low' (1.5) pH, deaminative cleavage occurs between GlcNS-GlcA and GlcNS-IdoA, albeit at a slower rate at the higher pH. The deamination reaction, and therefore chain cleavage, is regardless of O-sulfation carried by either monosaccharide unit.

At low pH, deaminative cleavage results in the release of inorganic SO4, and the conversion of GlcNS into anhydromannose (aMan). Low-pH nitrous acid treatment is an excellent method to distinguish N-sulfated polysaccharides such as heparin and HS from non N-sulfated polysacchrides such as chondroitin sulfate and dermatan sulfate, chondroitin sulfate and dermatan sulfate not being susceptible to nitrous acid cleavage.

The anticoagulant activity of heparin is primarily mediated through its binding and regulation of antithrombin , which is a serine protease inhibitor that inactivates various activated coagulation serine proteases, including factors, and thrombin The ability of AT to inhibit serine proteases is markedly enhanced in the presence of heparin. Accordingly, the interaction between heparin and AT is a crucial step in the anticoagulation process, which is the key step for the measurement of anticoagulant activity of heparin.

The chromogenic methods for anti-Xa and anti-IIa assay using commercial kits involve a two-step chromogenic method based on the inhibition of a constant, excess amount of factor Xa/IIa, by the tested heparin in presence of exogenous AT (stage 1), and the hydrolysis of factor Xa/IIa-specific chromogenic substrate, by residual factor Xa/IIa (stage 2). The p-nitroaniline (pNA) chromogen is then released from the substrate and the released amount is related to the residual factor Xa/IIa activity. The reactions for the two-step chromogenic method factor Xa activity are:

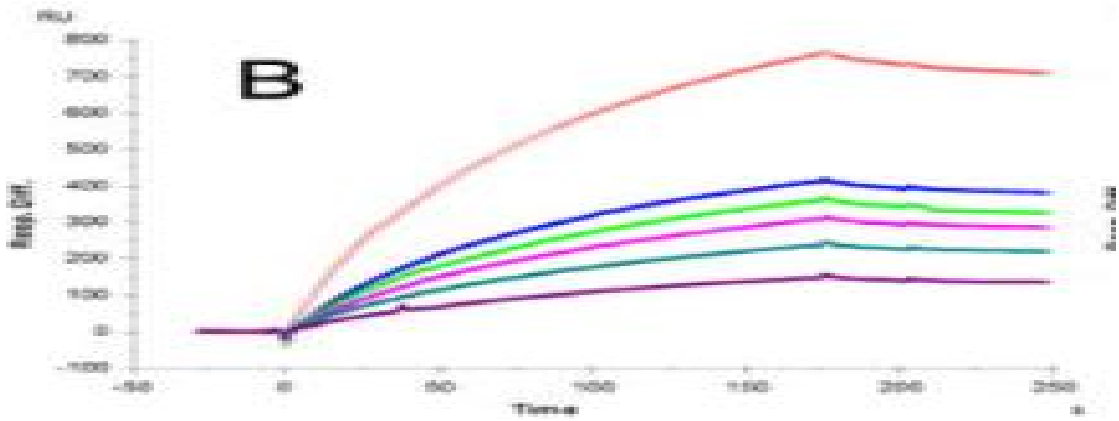
Stage 1: Hep. + AT → [AT Hep.];

Stage 2: [AT Hep.] + [FXa (excess)] → [FXa-AT-Hep.] + [residual FXa]; [residual FXa] + Substrate → Peptide + pNA (A405 nm)

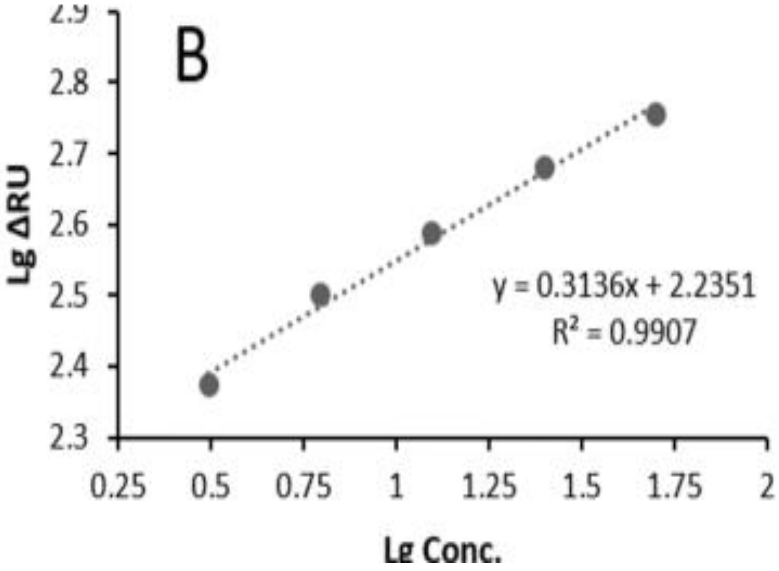
There is an inverse relationship between the concentration of heparin and color development, measured at 405 nm. These chromogenic methods require a high level of skill, are time consuming, and their accuracy can be impacted by the variable quality of available reagents.

This is a rapidly developing technique for research on molecular interactions, is label-free, real-time, medium-throughput and requires only small quantities of reagents. It uses an optical method to measure the change in refractive index of the medium close to a metal surface to monitor the binding of analyte molecules to receptor molecules, which are immobilized on the metal surface . We found that the binding between heparin and AT is easy to measure using SPR and this can be developed as a new method to quickly evaluate the anticoagulant activity of heparin.

The new method for the evaluation of Heparin includes a 20 µL solution of the heparin-biotin conjugate in running buffer [0.01 M Phosphate buffer 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20, (pH 7.4)] is injected over the flow cell at a flow rate 10 µL/min.



IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, December 2025
Impact Factor: 7.67



II. CONCLUSION

LMWH is a polymer with a molecular weight ranging from 3 to 30 kDa, although the average molecular weight of most commercial heparin preparations is in the range of 12 to 15 kDa.Heparin is a member of Carbohydrates is synthesized and validated by HPLC.