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“GREENING” OF THE INDUSTRIAL TECHNOLOGY OF ENOXAPARIN SODIUM SYNTHESIS

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The aim: *carrying out stages of synthesis of intermediates of Enoxaparin sodium, considering the variation of synthesis parameters. Correlation analysis between the technological parameters of the synthesis and the quality of the obtained samples. Evaluation of the influence of the quality of semi-finished products on the quality of the final substance. Implementation of the principles of green chemistry in the synthesis of Enoxaparin sodium by reducing the production cycle and using the most environmentally friendly solvents and reagents.*

Materials and methods: *samples of intermediates of the substance Enoxaparin sodium were synthesized according to the method described in the patent, as well as with a variation of the selected critical technological parameters. The obtained samples of intermediate products were analyzed according to the internal specification. In addition, an NMR-spectroscopy analysis was carried out for detailed structural characterization of Enoxaparin sodium intermediate molecules.*

Results*: the method of synthesis of intermediates of the Enoxaparin substance proposed in the patent was evaluated and the critical parameter for the formation of the final molecule was selected, namely the reaction mass holding time. The intermediates of Enoxaparin sodium – benzethonium salt of heparin and benzyl ester of heparin were developed according to the selected parameters and the analysis of the obtained samples was carried out according to the internal ND. Taking into account the principles of green chemistry, the method of synthesis of the intermediate product – benzyl ester of heparin was optimized by solvent regeneration.*

Conclusions*: As a result of the research, the methods of synthesis of intermediates of Enoxaparin were evaluated and the technological parameters of the synthesis of intermediates were determined, allowing to obtain a substance equivalent to the original Clexane® and Lovenox®. Evaluation of the method of synthesis of intermediate products according to the principles of green chemistry was carried out. The possibilities of greening the synthesis were analyzed. The holding time of the reaction mass of the benzethonium salt of heparin was reduced from 6 to 4 hours, and the benzyl ester of heparin from 25 to 22 hours. The E-factor indicator was reduced by regeneration of the solvent at the stage of synthesis of heparin benzyl ester*

Keywords*: enoxaparin, low molecular weight heparin, technological parameters, benzethonium salt of heparin, benzyl ester of heparin, green chemistry, e-factor, regeneration of solvents*

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1. Introduction

Enoxaparin sodium preparations, which are semi-synthetic analogues of sodium heparin, are extremely important and necessary drugs in the modern world for the prevention and treatment of disorders of the hemostasis system. Anticoagulants, in particular Enoxaparin, have gained critical importance with the emergence of the coronavirus pandemic. Enoxaparin sodium demonstrated a reduction in mortality due to the disease due to the normalization of hemodynamic parameters and an anti-inflammatory effect, which is manifested in the modulation of the body’s immune response to the stimulus [1, 2]. It was because of the outbreak of the pandemic that the need to increase the production of Enoxaparin increased, which became the impetus for the intensification of research and development of this active pharmaceutical ingredient.

The synthesis of Enoxaparin sodium molecule is a multi-stage process consisting of several transformations of the raw material sodium heparin under controlled conditions with the formation of intermediate products – benzethonium salt of heparin and benzyl ester of heparin. The synthesis and establishment of equivalence of generic products is a potentially complex phenomenon that requires high-precision technology to ensure analytical characterization and a positive result in preclinical studies and immunogenic evaluation [3]. That is why a necessary stage in the development of the method of synthesis of Enoxaparin sodium is the assessment of the methods of synthesis of intermediate products, the quality of which can potentially affect the quality of the final substance.

The benzethonium salt is formed by the exchange reaction of sodium heparin and a quaternary ammonium salt, in this case benzethonium chloride, followed by washing and drying of the intermediate. The following intermediate product is synthesized from the obtained benzethonium salt – heparin benzyl ester. It is formed due to an esterification reaction with the participation of an alkylating agent – benzyl chloride, followed by precipitation with a methanol solution of sodium acetate [4, 5]. The formation of the molecule is directly affected by the stage of alkaline hydrolysis of heparin benzyl ester, that is, the stage of chemical β-elimination. It requires high-precision control of technological indicators, as the synthesis conditions affect the composition of the molecule. The results of the study of the influence of technological parameters on the formation of Enoxaparin were described in the previous article [6]. The last step in a series of preliminary transformations is the purification of crude enoxaparin sodium by reprecipitation in methanol. It is known that the amount of methanol for reprecipitation of Enoxaparin affects the occurrence of certain modifications in the structure of the molecule, namely, it affects the distribution of short and long saccharide chains.

A complex technological process involves the use of many reagents and solvents, which, in turn, affects the environmental friendliness of the synthesis.

Since the 1940s, environmental problems began to arise in connection with the increase in industrial activity. The growing concern about the production of a large amount of waste and harmful substances in the chemical industry has led to the development of a whole field of modern science and technology, which is gaining more and more rapid development in our present [7]. Green chemistry is a production concept aimed at efficient use of raw materials and resources, avoidance of toxic and dangerous reagents, reduction of waste and by-products, including through their processing and regeneration with subsequent reuse [8]. All these implementations are aimed not only at reducing the burden on the environment, but also at improving the economic competitiveness of production.

The year 1990 is considered the official beginning of the development of this industry, when, in connection with the growth of environmental awareness, the American Environmental Protection Agency (EPA) introduced the pollution prevention law [9]. Thanks to this law and many publications of scientists, 12 Principles of green chemistry were formed, which became a kind of reference point in the planning and optimization of chemical production technologies [10, 11].

To simplify the implementation of “green” technologies, metrics were developed that easily allow understanding the conditional degree of environmental friendliness. Below are the main ones:

1. E-factor – the ratio of the amount of waste to the amount of product. In the calculation of the E-factor, all used raw materials, except water, are included in the calculation.
2. PMI (Process mass intensity) is a metric similar to the E-factor, but it is calculated by the ratio of the mass of all materials used to the mass of the final product.
3. Atom Economy – the ratio of the relative molecular mass of the final product to the relative molecular mass of all reagents, expressed as a percentage [12, 13].

In the pharmaceutical industry, solvents make up 80–90 % of the mass of materials used for the synthesis of APIs. They play a dominant role in the toxicity profile of any process [14]. In 2012, the public-private partnership Innovative Medicines Initiative CHEM21 was established, which included pharmaceutical companies, universities, and small and medium-sized enterprises. This partnership has developed guidelines for solvent selection, each of which includes a list of evaluation criteria (environmental impact criteria, safety criteria, etc.) that sort solvents into the following categories: [15, 16]:

– recommended (or preferred): solvents to be tested first in a screening exercise, if of course there is no chemical incompatibility in the process conditions.

– problematic: these solvents can be used in the lab or in the Kilolab, but their implementation in the pilot plant or at the production scale will require specific measures, or significant energy consumption.

– hazardous: the constraints on scale-up are very strong. The substitution of these solvents during process development is a priority.

– highly hazardous: solvents to be avoided, even in the laboratory [17].

The aim of this work was to monitor the possible dependence of the quality indicators of semi-finished products on the technological parameters of their synthesis, as well as to assess the influence of the quality of semi-finished products on the quality of the final substance. The task was also to implement the principles of green chemistry in the synthesis of Enoxaparin sodium, which involves reducing the production cycle and using the most environmentally friendly solvents and reagents.

2. Research planning (methodology)

The research was based on the synthesis method described in the patent [4].

The research included a step-by-step development of the research goal:

1. The first stage of this work is to evaluate the possibilities of “greening” the synthesis of intermediates by varying the parameters of individual stages given in the patent:

1. 1. The intermediate benzethonium salt of heparin is obtained in an aqueous environment and at room temperature, which does not contradict the principles of green chemistry and increases the energy efficiency of the process. The technological parameter that can be considered as critical and the one that affects the “greening” of the synthesis is the indicator of the holding time of the reaction mass. Reducing the holding time will allow to reduce the production cycle and reduce the energy consumption of production. Since the patent does not describe the reaction mass holding time in the process of producing benzethonium salt of heparin, this parameter and its variation were chosen empirically in the range of 6±2 hours.

1. 2. At the stage of obtaining the benzyl ester of heparin, we paid attention to the possibility of variation in the time of esterification, and considered the solvents used for the synthesis, since esterification takes place with the participation of reagents that significantly affect the safety and environmental friendliness of the synthesis.

As stated in the patent, the partial ester of heparin in salt form, the degree of esterification of which ranges from 9.5 % to 14 %, may be prepared by esterification of the long-chain quaternary ammonium salt of heparin in a chlorinated organic solvent, in the presence of a chlorine derivative. Preferably, the chlorine derivative is benzyl chloride, and the chlorinated solvent is either chloroform or methylene chloride [4].

In other patents, there is also information about the possibility of using other solvents:

For the esterification (step-b), the inert solvent used for dissolving the heparin benzethonium salt is selected from N, N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAc), methylene chloride (DCM) and the like [18].

Solvents should be considered to increase the "greening" of the synthesis of heparin benzyl ester:

– dichloromethane is an organochlorine solvent, a toxic, volatile compound;

– chloroform is an organochlorine solvent, depresses the central nervous system, and is a probable carcinogen;

– dimethylacetamide is an aprotic solvent, a flammable, combustible substance that exhibits reproductive toxicity;

– dimethylformamide is an aprotic solvent, a substance with reproductive toxicity;

– methanol is a representative of monoatomic alcohols, a poisonous substance.

The choice between CHCl$_3$/DCM/DMF/DMA was made by us based on the evaluation of data in the guidelines for the selection of solvents developed by global pharmaceutical companies [15]. As indicated in Table 1, dichloromethane has an advantage in use compared to other solvents. In addition, it is worth noting that dichloromethane is the most popular chlorinated solvent in terms of frequency of use. This is also due to its low cost, which creates an advantage in terms of reducing production costs [19].

In addition, the European Regulation on the "Registration, Evaluation, Authorization and Restriction of Chemicals" (REACH) has imposed restrictions on chloroform and dichloromethane with certain conditions, while DMF and DMAc are listed as "substances of very serious concern" (SVHC) [20, 21].

Replacing methanol, as a poisonous substance, with a safer solvent at this stage is impossible due to the physical and chemical properties of sodium acetate, the solution of which is necessary for precipitation of the benzyl ester of heparin. For example, the solubility of sodium acetate in methanol is 16 g/100 g (15 °C), while in ethanol only the trihydrate form is soluble in the amount of 5.3 g/100 ml. Thus, the replacement of solvents at this stage can significantly affect the yield and quality of heparin benzyl ester. Therefore, experimental studies were carried out using dichloromethane, other solvents and reagents were used according to the patent.

Table 1

Generalized rating comparison of solvents

Solvent	Astra Zeneka	GCI-PR	Glaxo Smith Klein	Pfizer	Sanofi	Overall
DCM	20	18	5	Undesirable	Substitution advisable	To be confirmed (problematic or hazardous)
CHCl$_3$	–	18	4	Undesirable	Banned	Highly hazardous
DMF	20	17	7	Undesirable	Substitution requested	Hazardous
DMAc	20	16	4	Undesirable	Substitution requested	Hazardous

The selected parameter for possible "greening" at this stage is a variation of the holding time in the range of 25±3 hours.

2. At the second stage, carry out a gradual synthesis/purification of intermediates products according to the conditions specified in the patent and the planned variation of parameters, a comparison of their quality indicators and an assessment of the influence of the quality of intermediates on the quality of the final product.

3. According to the results of the conducted experimental studies, make corrections to the general method of synthesis of Enoxaparin sodium.

3. Materials and methods

This study was conducted during 2019–2021.

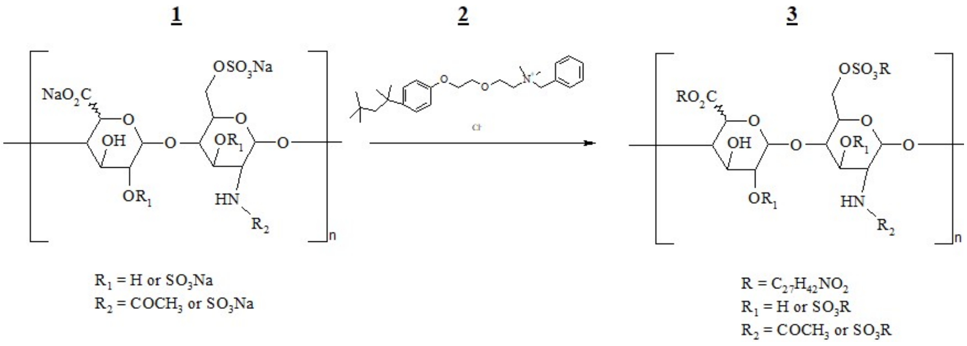
Water test was carried out on Mettler Toledo T70 titrator (Switzerland) (*Ph. Eur. 2.5.12*), Sodium test was made on High-Resolution Continuum Source Atomic Absorption Spectrometer for Flame and Hydride Analytik Jena Contr AA 300 (Germany) (*Ph. Eur. 2.2.23*), Loss on drying test was performed on Pol-Eko Aparatura slw 53 (*Ph. Eur. 2.2.32*). Assay – free benzyl alcohol test was carried out by HPLC method on Agilent 1260 Infinity II (USA), column Zorbax SB-C18 (150 mm*4.6 mm*5 µm) (*Ph. Eur. 2.2.29, 2.2.46*), residual solvents test was performed by a method of headspace gas chromatography on Agilent GC 7890B (USA), column DB – 624 sized 60 m×0.32 mm, with a layer thickness of 1.8 µm (*Ph. Eur. 2.2.28, 2.2.46*). Molecular Weight Distribution and Weight-Average Molecular Weight was performed on Shimadzu chromatograph (Japan), column PX_TSKgel G2000SW (300 mm*7.8 mm*5 mkm) with detector module Viscotec 305, Malvern Instruments LTD (England) (*Ph. Eur. 2.2.30*)

3. 1. Preparation of benzethonium salt of heparin

A solution of benzethonium chloride **2** (25 g) in water (125 ml) was added to a solution of heparin sodium **1** (10 g) prepared in water (100 ml). The product obtained **3** was then filtered off, washed with water and thereafter dried.

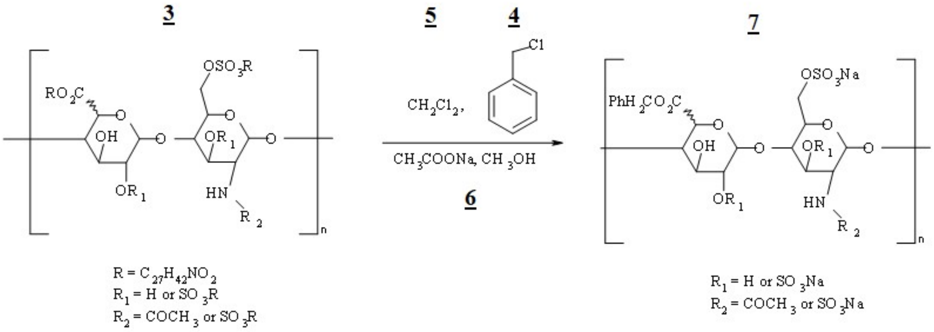
The yield of the intermediate product is 98 % of the theoretical yield.

The samples of the benzethonium salt of heparin, which were processed at the first stage according to the given methodology and with variations of the "holding time" parameter, were analyzed according to internal regulatory documents. The *"Water"* indicator was determined according to the *Ph. Eur. 2.5.12*, it was chosen to control the drying efficiency of the intermediate product, since the next stage takes place in anhydrous conditions. The water content criterion "no more than 2.0 %" was determined statistically. Such a content does not interfere with the course of the next reaction and is optimal in view of the physical characteristics of the intermediate product. Separately from the regulatory document, the obtained samples were analyzed to obtain informative data on the *"Sodium"* indicator (*Ph. Eur. 2.2.23*), which controls the quality of washing the benzethonium salt of heparin from sodium chloride, which is formed as a by-product in the reaction process. In addition, the Ronzoni Institute experimentally conducted an analysis of the proof of the structure of molecules by the method of proton NMR ^1H spectroscopy: the solvent is $CDCl_3$.



3. 2. Preparation of benzyl ester of heparin

Benzyl chloride **4** (15 ml) was added to a solution of benzethonium heparinate **3** (15 g) in methylene chloride **5** (75 ml). The solution was heated to a temperature of 35 °C, which was maintained for 25 hours. A 10 % solution (90 ml) of sodium acetate in methanol **6** was then added, the mixture was filtered, and the product was washed in methanol and dried. Heparin benzyl ester **7** (6.5 g) was thereby obtained in the form of a sodium salt, the degree of esterification of which, determined as described above, was 13.3 %.



The samples were processed according to the described method with a variation of the "holding time" indicator in the range of ±3 hours, i.e., the esterification reaction lasted 22, 25, and 28 hours. The development of benzyl ester took place with a certain difference from the patent at the stage of isolation of the intermediate product. After precipitation with a methanol solution of sodium acetate, a sticky amorphous precipitate is formed, which has a sharp smell of benzyl alcohol. Therefore, a necessary step is the purification of the intermediate, which was separated into a separate step, described below. Thus, after carrying out the esterification reaction and isolating the crude benzyl ester of heparin, the filtered but not dried intermediate is dissolved in an aqueous solution of sodium chloride with subsequent precipitation in methanol.

Analysis and evaluation of the quality of intermediate products took place after the purification of the benzyl ester of heparin.

3. 3. Purification of heparin benzyl ester

Add sodium chloride (0.09 kg) to purified water (0.79 kg). Add the filtered benzyl ester of heparin to the sodium chloride solution, mix until a homogeneous mass is formed. Add the resulting mixture to methanol (1.4 kg). The mixture was filtered, and the product was washed in methanol and dried.

Samples of purified benzyl ester of heparin were analyzed according to the internal specification of JSC Pharmak according to the indicators *"Residual amounts of organic solvents"* (*GC, Ph. Eur. 2.2.28, 2.2.46*) and *"Loss in mass during drying"* (*Ph. Eur. 2.2.32*) – as criteria for the quality of drying of the intermediate product, *"Quantitative determination - free benzyl alcohol"* (*HPLC, Ph. Eur. 2.2.29, 2.2.46*) – to understand the quality of washing of the intermediate product. As an experiment, the analysis was carried out according to the parameters *"Sodium"* (*AAC, Ph. Eur. 2.2.23*) and *"Average relative molecular weight"* (*Ph. Eur. 2.2.30*) – to understand the dynamics of the change in molecular weight during the reaction.

The structure was confirmed by ^1H NMR by the Ronzoni Institute: D_2O solvent, TSP internal standard.

A factor that can green the synthesis at this stage is the regeneration of methanol for its reuse.

4. Research results and their discussion

4. 1. Preparation of benzethonium salt of heparin

At the first stage, the benzethonium salt of heparin 3 is obtained, which is formed by mixing solutions of benzethonium chloride 2 and sodium heparin 1. At this stage, the substances react quantitatively, which prevents the formation of by-products.

For comparison, we developed and analyzed samples with the following variations in holding time:

- – 4 hours (D 461);
- – 6 hours (D 462);
- – 8 hours (D 460).

It was noted that the variation of the reaction mass holding time did not affect its yield. An analysis of the processed samples was carried out according to the internal processes (Table 2). The results of the analysis meet the requirements of regulatory documentation.

For a more detailed study of the composition of the obtained intermediates and to identify possible differences, an analysis of the benzethonium salt of heparin by the ^1H NMR method was carried out (Fig. 1).

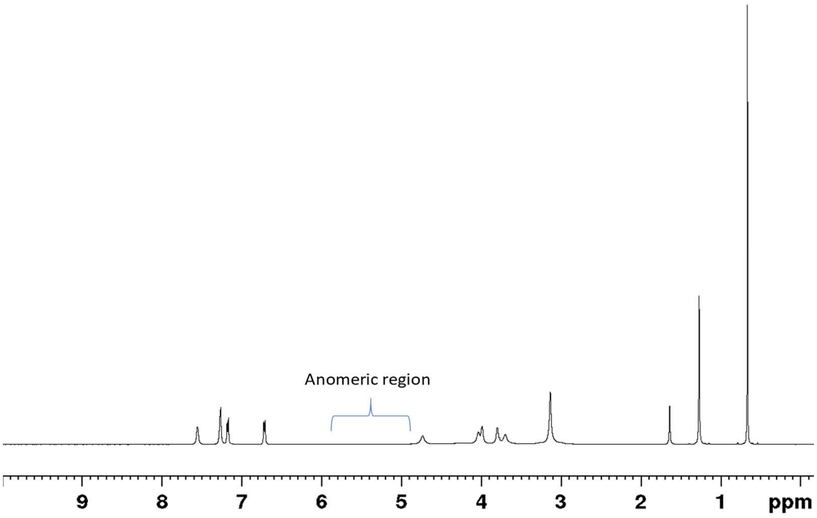


Fig. 1. Proton NMR spectrum of the benzethonium salt of heparin

Table 2

Results of analysis of samples of benzethonium salt of heparin

Indicator	Requirements of ND	D461	D462	D460
Water, %	Not more than 2.0 %	1.8	1.7	1.8
Sodium, %	Information quantity	<LOD	<LOD	<LOD

4. 2. Preparation of benzyl ester of heparin

Samples of heparin benzyl ester were developed with a variation of the technological parameter “holding time” to shorten the production cycle, which contributes to the “greening” of the synthesis. So, the esterification time varied as follows:

– 22 hours (D 463);

– 25 hours (D 464 – according to the patent US 5,389,618);

– 28 hours (D 465).

The quality assessment of the obtained experimental samples was carried out according to the internal specification.

As shown by the results of the experimental determination of quality parameters, all synthesized samples demonstrated compliance with the requirements of the internal ND (Table 3).

The next stage was the study of the structure of the synthesized samples of heparin benzyl ester by the ^{1}H NMR method. The comparative spectrum of samples is presented in Fig. 2, 3. The obtained spectra were evaluated and compared based on chemical shifts and integral signal intensity of benzyl protons and heparin residue (Fig. 3).

Table 3

Results of the analysis of heparin benzyl ester samples

Indicator	Requirements of ND	D 463	D 464	D 465
Loss in mass during drying, %	Not more than 10.0 %	7.5	7.1	7.6
Sodium, %	Information quantity	8.6	8.6	9.7
Quantitative determination (free benzyl alcohol), %	Not more than 0.1 %	0.01	0.02	0.01
Residual amounts of organic solvents, ppm	Methanol NMT 3000 ppm	189	1073	434
	Dichloromethane NMT 600 ppm	0	0	0
Identification (Average relative molecular weight)	Information quantity	17472	17558	18009

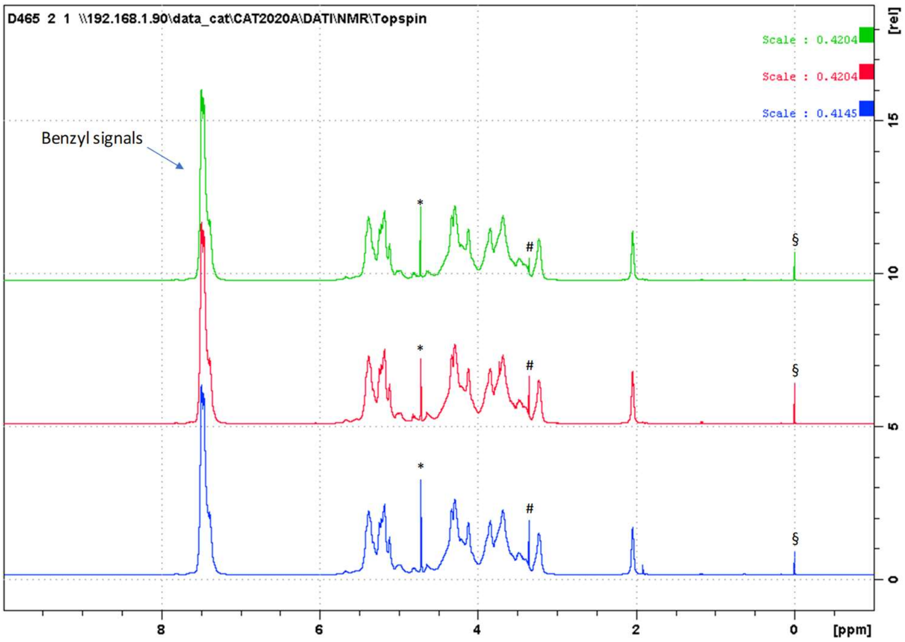
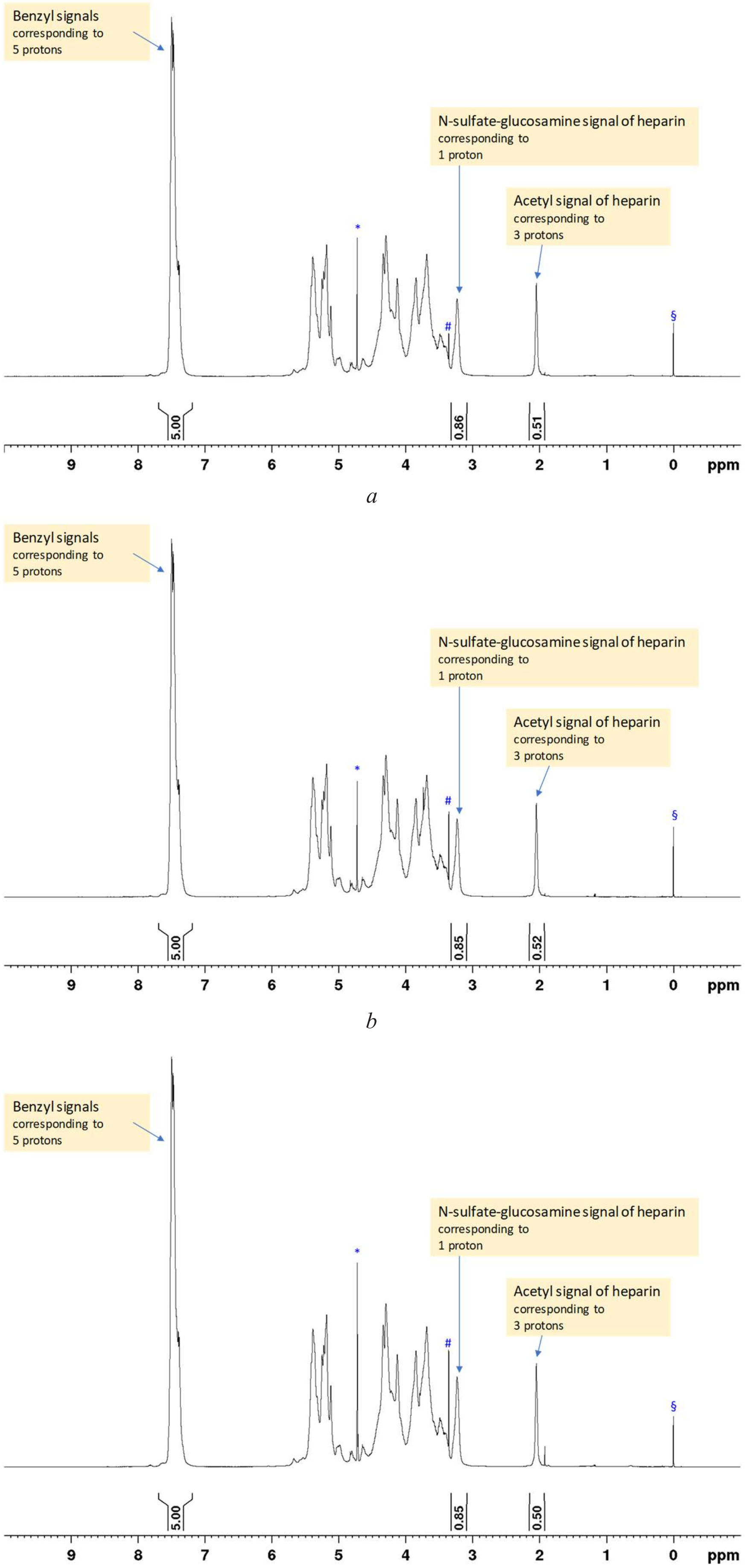


Fig. 2. Proton NMR spectrum of heparin benzyl ester: HOD residual signal, # methanol, § TSP D.463 (green), D464 (red) D465 (blue)



Benzyl signals corresponding to 5 protons

N-sulfate-glucosamine signal of heparin corresponding to 1 proton

Acetyl signal of heparin corresponding to 3 protons

a

Benzyl signals corresponding to 5 protons

N-sulfate-glucosamine signal of heparin corresponding to 1 proton

Acetyl signal of heparin corresponding to 3 protons

b

Benzyl signals corresponding to 5 protons

N-sulfate-glucosamine signal of heparin corresponding to 1 proton

Acetyl signal of heparin corresponding to 3 protons

c

Fig. 3. Proton NMR spectrum and the corresponding integrals: *a* – D463; *b* – D464; *c* – D465

4. 3. Purification of heparin benzyl ester

This stage is not described in the patent but is necessary because it allows you to obtain an intermediate product that does not contain solvent residues (dichloromethane) and by-products formed as a result of the reaction, for example, benzyl alcohol.

The purification stage of the benzyl ester involved the regeneration of methanol.

The regeneration yield was 70 %. The calculation of the E-factor considering the regeneration of methanol is given in Table 4.

The obtained E-factor value without methanol regeneration is 131.4.

Recalculation of the E-factor considering methanol regeneration – 68.4.

Table 4

Calculation of the E-factor of the stage of obtaining the benzyl ester of heparin, taking into account the regeneration of the solvent

Materials	Mass of materials, kg		Mass of product, kg	E-factor	E-factor with solvent recovery
	without solvent recovery	solvent recovery			
Benzethonium heparinate	0.44	0.44	0.1	131.4	68.4
DCM	2.93	2.93			
Benzylchloride	0.48	0.48			
Sodium acetate	0.22	0.22			
Sodium chloride	0.17	0.17			
Methanol	9.0	2.7			
13.24		6.94			

4. 4. Obtaining crude Enoxaparin sodium

The synthesis stage of Enoxaparin sodium was studied separately and described earlier [4]. As a result of the work carried out, the technological parameters of the Enoxaparin synthesis process were optimized – the temperature of the reaction mass was reduced from 62 °C to 57 °C, which positively affects the greening of the synthesis by reducing energy consumption. Regeneration of methanol at the stage of obtaining technical Enoxaparin sodium is impossible, because the mother liquor contains many degradation products of the depolymerization reaction, which makes it impossible to obtain a solvent of appropriate purity after distillation.

4. 5. Purification of technical enoxaparin sodium

Development and improvement of the purification technique of crude Enoxaparin sodium is planned as an object of further research.

5. Discussion of research results

5. 1. Preparation of benzethonium salt of heparin

Analysis and proof of the structure of heparin derivatives is a difficult task due to the heterogeneous molecular structure and large molecular weight. Scientists use the ^{1}H NMR spectroscopy method, which is the most common and very informative, to analyze the structures of the glycosaminoglycan family, which includes Enoxaparin and its intermediates [22, 23]. So, for extended control, it was decided to analyze the obtained intermediates by this method.

At this stage of the synthesis, we compared the sample obtained under the conditions specified in the patent and the samples developed with a variation of the "holding time" parameter to assess the dynamics of changes in the structure and quality of the intermediates product. The analysis of the obtained samples was carried out according to the internal procedures. As can be seen from the obtained results (Table 2), all experimental samples meet the quality parameters established by the regulatory documentation.

For additional control, the Ronzoni Institute conducted a study of the structure of the obtained samples using ^{1}H NMR spectroscopy.

On the PMR spectrum (Fig. 1) there is a signal belonging to the benzethonium component, but there is no signal belonging to the anomer of heparin. Thus, the PMR spectroscopy method is uninformative for assessing the quality of this intermediate product.

After the general assessment of quality, it is possible to conclude that the influence of the time of holding of the reaction mass during the synthesis of benzethonium salt of heparin is not critical. Since in view of the principles of "green chemistry" preference is given to shortening the technological process, the smallest number of hours of holding (D 461) was chosen for further work at this stage.

5. 2. Preparation of benzyl ester of heparin

The stage of obtaining the benzyl ester of heparin is more complicated than the previous one in view of the type and number of reagents used for synthesis. We conducted an assessment and selected the solvent with the most environmentally friendly properties. Also, the parameter to be varied was selected and the developed samples with holding times of 22, 25 and 28 hours were analyzed according to the internal specification. The analysis of the obtained intermediates was carried out after the purification of the processed samples to obtain correct results. According to the results of the analysis according to procedures, the obtained samples meet the requirements, which indicates the possibility of reducing the reaction mass holding time without changing the quality of the intermediate product (Table 3).

In addition, an analysis by the ^{1}H NMR spectroscopy method conducted by the Ronzoni Institute was involved (Fig. 2, 3)

Special attention was paid to the ratio of heparin and benzyl protons, which indicates the completeness of the reaction. The molar ratio between heparin and the benzyl group was determined by the integration of specific proton signals: the signal of the methyl group of the acetyl fragment of heparin at 2.04 ppm (N-acetylglucosamine) and the proton signal at 3.23 ppm (N-sulfate-glucosamine), integrated against the signal of aromatic of benzyl protons at 7.7–7.2 ppm. According to the structure of the molecule, we expect that 5 protons belong to the integral of the benzyl group, 3 protons to the integral of

CH_3 – the group of the acetyl fragment, and 1 proton to H in the 2 position of N-sulfate-glucosamine.

Each integral value of the three spectra (Fig. 3) was divided by the number of protons, and the resulting value was used to calculate the heparin/benzyl ratio as follows:

$$\left(\begin{array}{l}\text{Acetyl integral}/3+\\ +\text{ANS integral}/1\end{array}\right)/\left(\text{Benzyl integral}/5\right)=$$
$$=\text{heparin}/\text{benzyl ratio}.$$

According to the proton NMR spectrum, the samples obtained at different holding times show the same heparin/benzyl ratio – 1.02–1.03. Since the indicators of the heparin/benzyl molar ratio are almost the same, this indicates that there is no influence of the reaction mass holding time in these ranges on the quality of the intermediate. Thus, it can be argued that reducing the holding time of the above-described stage to 22 hours allows obtaining a intermediate product of appropriate quality and reducing the production cycle, which corresponds to the principles of green chemistry in energy saving.

5. 3. Purification of heparin benzyl ester

We managed to significantly reduce the E-factor by regenerating the methanol obtained at the stage of purification of the benzyl ester of heparin. The regeneration yield was 70 %, which made it possible to reduce the E-factor from 131.4 to 68.4.

Study limitations. The limitation is that a larger variation of the parameters can negatively affect the characteristics of the Enoxaparin sodium substance. Also, increasing the variation of technological parameters during the development of synthesis technology usually requires additional resources in terms of personnel, materials, and time.

Prospects for further research. It is planned to investigate and optimize the method of purification of crude Enoxaparin sodium from the point of view of composition and principles of green chemistry.

6. Conclusions

As a result of the conducted experiments, the optimal technological parameters for the synthesis of Enoxaparin sodium intermediates were established from the point of view of the quality of the final product and the principles of green chemistry. Since the final goal of the research was the synthesis of Enoxaparin sodium, equivalent to the original Clexane® and Lovenox®, it was established that changing the selected parameter, namely the time of reaction mass holding in the above-described ranges during the synthesis of intermediate products, does not affect the quality and composition of the Enoxaparin molecule, therefore we chose the parameters that contribute to the greening of the technique.

Thus, the reaction mass holding time at the stage of heparin benzethonium salt formation was reduced from 6 to 4 hours, which made it possible to shorten the production cycle. At the stage of formation of heparin benzyl ester, without changing the quality of the intermediate product, the reaction mass holding time was reduced from 25 to 22 hours, which also reduced the production cycle and energy costs. Methanol regeneration was successfully implemented at the stage of heparin benzyl ester purification, thanks to which it was possible to reduce the E-factor from 131.4 to 96.4.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

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References

1. Billett, H. H., Reyes-Gil, M., Szymanski, J., Ikemura, K., Stahl, L. R., Lo, Y. et al. (2020). Anticoagulation in COVID-19: Effect of Enoxaparin, Heparin, and Apixaban on Mortality. Thrombosis and Haemostasis, 120 (12), 1691–1699. doi: https://doi.org/10.1055/s-0040-1720978

2. Drago, F., Gozzo, L., Li, L., Stella, A., Cosmi, B. (2020). Use of Enoxaparin to Counteract COVID-19 Infection and Reduce Thromboembolic Venous Complications: A Review of the Current Evidence. Frontiers in Pharmacology, 11. doi: https://doi.org/10.3389/fphar.2020.579886

3. Iqbal, Z., Sadaf, S. (2022). Commercial Low Molecular Weight Heparins – Patent Ecosystem and Technology Paradigm for Quality Characterization. Journal of Pharmaceutical Innovation, 18 (2), 803–835. doi: https://doi.org/10.1007/s12247-022-09665-7

4. Mixtures of particular LMW heparinic polysaccharides for the prophylaxis/treatment of acute thrombotic events (1995). Pat. US 5,389,618. 14.02.1995.

5. Baytas, S. N., Linhardt, R. J. (2020). Advances in the preparation and synthesis of heparin and related products. Drug Discovery Today, 25 (12), 2095–2109. doi: https://doi.org/10.1016/j.drudis.2020.09.011

6. Bovsunovska, Y., Rudiuk, V., Mishchenko, V., Georgiyants, V. (2023). Obtaining the substance enoxaparin sodium equivalent to the original Clexane® and Lovenox®. Selection of technological parameters of the key stage of the synthesis. ScienceRise: Pharmaceutical Science, 2 (42), 46–56. LOCKSS. doi: https://doi.org/10.15587/2519-4852.2023.277735

7. de Marco, B. A., Rechelo, B. S., Tótoli, E. G., Kogawa, A. C., Salgado, H. R. N. (2019). Evolution of green chemistry and its multidimensional impacts: A review. Saudi Pharmaceutical Journal, 27 (1), 1–8. doi: https://doi.org/10.1016/j.jsps.2018.07.011

8. Sheldon, R. A. (2017). Metrics of Green Chemistry and Sustainability: Past, Present, and Future. ACS Sustainable Chemistry & Engineering, 6 (1), 32–48. doi: https://doi.org/10.1021/acssuschemeng.7b03505

9. Sheldon, R. A. (2017). The E factor 25 years on: the rise of green chemistry and sustainability. Green Chemistry, 19 (1), 18–43. doi: https://doi.org/10.1039/c6gc02157c

10. Ivanković, A. (2017). Review of 12 Principles of Green Chemistry in Practice. International Journal of Sustainable and Green Energy, 6 (3), 39–48. doi: https://doi.org/10.11648/j.ijrse.20170603.12

11. Wanisa, A. M., Qasem, A. A., Asma, O. E. (2020). Green chemistry: principles, applications, and disadvantages. Chemical Methodologies, 4, 408–423. doi: https://doi.org/10.33945/sami/chemm.2020.4.4

12. Gupta, P., Mahajan, A. (2015). Green chemistry approaches as sustainable alternatives to conventional strategies in the pharmaceutical industry. RSC Advances, 5 (34), 26686–26705. doi: https://doi.org/10.1039/c5ra00358j

13. Dunn, P. J. (2012). The importance of Green Chemistry in Process Research and Development. Chemical Society Reviews, 41 (4), 1452–1461. doi: https://doi.org/10.1039/c1cs15041c

14. Constable, D. J. C., Jimenez-Gonzalez, C., Henderson, R. K. (2006). Perspective on Solvent Use in the Pharmaceutical Industry. Organic Process Research & Development, 11 (1), 133–137. doi: https://doi.org/10.1021/op060170h

15. Prat, D., Hayler, J., Wells, A. (2014). A survey of solvent selection guides. Green Chemistry, 16 (10), 4546–4551. doi: https://doi.org/10.1039/c4gc01149j

16. Alder, C. M., Hayler, J. D., Henderson, R. K., Redman, A. M., Shukla, L., Shuster, L. E., Sneddon, H. F. (2016). Updating and further expanding GSK's solvent sustainability guide. Green Chemistry, 18 (13), 3879–3890. doi: https://doi.org/10.1039/c6gc00611f

17. Prat, D., Wells, A., Hayler, J., Sneddon, H., McElroy, C. R., Abou-Shehada, S., Dunn, P. J. (2016). CHEM21 selection guide of classical- and less classical-solvents. Green Chemistry, 18 (1), 288–296. doi: https://doi.org/10.1039/c5gc01008j

18. Process for the preparation of low molecular weight heparin (2018). Pat. WO2019116217A2.

19. Jordan, A., Stoy, P., Sneddon, H. F. (2020). Chlorinated Solvents: Their Advantages, Disadvantages, and Alternatives in Organic and Medicinal Chemistry. Chemical Reviews, 121 (3), 1582–1622. doi: https://doi.org/10.1021/acs.chemrev.0c00709

20. Byrne, F. P., Jin, S., Paggiola, G., Petchey, T. H. M., Clark, J. H., Farmer, T. J. et al. (2016). Tools and techniques for solvent selection: green solvent selection guides. Sustainable Chemical Processes, 4 (1). doi: https://doi.org/10.1186/s40508-016-0051-z

21. Zhenova, A. (2020). Challenges in the development of new green solvents for polymer dissolution. Polymer International, 69 (10), 895–901. doi: https://doi.org/10.1002/pi.6072

22. Rudd, T. R., Skidmore, M. A., Guimond, S. E., Cosentino, C., Torri, G., Fernig, D. G. et al. (2008). Glycosaminoglycan origin and structure revealed by multivariate analysis of NMR and CD spectra. Glycobiology, 19 (1), 52–67. doi: https://doi.org/10.1093/glycob/cwn103

23. Pomin, V. H., Mulloy, B. (2021). Nuclear Magnetic Resonance Methods in Structural Characterization of Glycosaminoglycans. Glycosaminoglycans. New York, 183–207. doi: https://doi.org/10.1007/978-1-0716-1398-6_16

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