# FIBROUS SYSTEMS WITH PROGRAMMED BIOLOGICAL-ACTIVITY AND THEIR APPLICATION IN MEDICAL PRACTICE

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## *Abstract*

*An effective two-stage method for obtaining both biologically activated fibres with antibacterial and anaesthetic activity and biologically activated complex fibres -insulin as an artificial store of insulin has been developed. The first stage involves the formation of reactive functional groups by chemical modification, followed by the second stage where the fibres are modified with chemotherapeutic agent solutions.*
*This paper presents the results of obtaining biologically activated fibres with antibacterial and anaesthetic activity as well as an artificial store of insulin in the form of complex ion-exchanged fibre-insulin. The level of immobilisation of the drug in the antibacterial fibre amounts to 140 mg of gentamicin sulphate per 1 g of fibres, in the anaesthetic fibre 180 mg of procaine hydrochloride per 1 g of fibres, and in the fibrous store of insulin 800 mg of insulin per 1 g of fibres.*

## Introduction

The use of fibres in medicine and surgery has been known from ancient times. In recent years, the application of fibres for medical purposes has been growing very rapidly. Fibrous systems have found a wide range of applications, for example in masks, napkins, sponges, bandages, surgical threads, glue for tissues, semi-permeable membranes, artificial organs, all kinds of implants, drug carriers, and many other areas[1,2].

Fibres with inherent biological activity are called biologically activated fibres. Such fibres are produced by the modification of chemical or natural fibres with chemotherapeutic agents either in polymer substrate or on the fibre surface. Biologically activated fibres suitable for a biomedical applications must be “biocompatible”, at least on the surface. Proper modification may lead to the formation of fibres with antibacterial, anti-fungal, anaesthetic, fermenting, radioactive, haemoactive (affecting blood coagulation) agents, and fibres with immobilised hormones (insulin, adrenaline and noradrenaline) [3,4].

Chemical fibres (mainly synthetic ones) have attained priceless importance in medical science. They are of great interest not only for their high chemical and mechanical resistance and moulding susceptibility, but also for their easy-to-control manufacturing and sterilisation processes. A large number of modifications in cellulose, polyacrylonitrile, polyamides, polyester, polyolefin and polyvinyl alcohol fibres have been made to impart various medical properties according to the requirements of biological systems.

Chemotherapeutic agents are made to react with reactive groups present in the fibre. In case of highly unreactive fibres as polyacrylonitrile, polyethylene and polyester, reactive groups are introduced by way of modification so that the chemotherapeutic agent can react to impart biological activity. Functional groups properly located on a fibre, as well as its structure, are usually responsible for its biocompatibility and/or biodegradability, and may impart either therapeutic or/and toxic characteristics to it. For example, carboxylic groups induce therapeutic activity in many drugs, and moderately improve the biocompability of the fibres [1].

The release of chemotherapeutic agents, absorbed or chemically bonded by fibres, involves their slow and controllable diffusion from or through fibrous materials. Controlled-release systems have significantly improved the delivery of many existing drugs, and offer a number of potential advantages

over conventional drug therapy. Controlled-release preparation can maintain the drug in the desired therapeutic range by means of a single dose. Other advantages of controlled release include: localised delivery of the drug to a particular body area, which lowers the systemic drug level; reduced need for follow-up care; maintenance of medications that are rapidly destroyed by the body; increased patient comfort; and improved patient compliance [5]. Recent research efforts have been concentrated on systems capable of directing drugs towards sick organs or cells. Such strategies seem to provide the most effective approach to drug therapy, and may represent one of the main trends of the future.

Our research is directed towards obtaining biologically activated fibres with antibacterial and anaesthetic activity, as well as obtaining biologically activated complex ion-exchanged fibres – insulin as an artificial store of insulin. The aim of the present research is to examine possibilities of obtaining the above-mentioned biologically activated fibres on the basis of polyacrylonitrile (PAN) fibres. PAN fibres were chosen for this purpose, above all, because of their good chemical stability, the presence of reactive nitrile groups and their high degree of porosity. Furthermore, PAN fibres are recognised as one of the most durable within living organism tissues. Because PAN fibres do not contain an appropriate amount of functional (acid) groups, the authors intended to create them by hydrolysis of nitrile groups by an aqueous sodium metasilicate solution, and obtaining the cation-exchanged acrylic fibres before incorporating the selected therapeutic agents.

## Experimental

### Materials

PAN fibre with linear density of 6.4 dtex, representing a terpolymer of acrylonitrile, methylacrylate and itaconic acid, was used for the present study. To obtain cation-exchanged PAN fibres by saponification of nitrile groups, as well as for their further modification, the following reagents were used:

- sodium metasilicate ($Na_2SiO_3$ x $5H_2O$), sodium hydroxide, hydrochloric acid, sulphuric acid,
- gentamicin sulphate, procaine hydrochloride, insulin from porcine pancreas (approx. 24 I.U. per mg, crystalline), acetate buffer, physiological solution.

### Methods

Obtaining cation-exchanged PAN fibres.

Carboxylic groups were introduced into PAN fibres by saponification of nitrile groups by aqueous sodium metasilicate solutions. The saponification was carried out under following conditions: $Na_2SiO_3$ concentration of 0.4-2%, modulus of the bath from 1:10 to 1:125, at 75-100$^oC$, for 1-90min.

Determination of carboxylic groups in cation-exchanged PAN fibres.

The amount of carboxylic groups was determined by the volumetric method, where fibres were treated with 0.01M NaOH at room temperature for 1 hour. After that, the solution was titrated with 0.01M HCl in the presence of an indicator.

Obtaining biologically activated fibres.

Biologically activated fibres with antibacterial activity were obtained by modifying cation-exchanged PAN fibres with a water solution of gentamicin sulphate under the following conditions: antibiotics concentration of 3.5-7x$10^{-3}$mol/$dm^3$, at pH 5.5, modulus of the bath 1:400, at 20±4$^oC$ for 1min–24 hours. The constant pH value of the solution was maintained by the addition of hydrochloric acid.

Biologically activated fibres with anaesthetic activity were obtained by modification of cation-exchanged PAN fibres with a water solution of procaine hydrochloride under the following conditions: anaesthetic concentration of 3.5x$10^{-3}$mol/$dm^3$, pH of 5, modulus of the bath 1:200, at 20±4$^oC$ for 15min-24 hours. The constant pH value of the solution was maintained by the addition of hydrochloric acid.

A biologically activated complex of ion-exchanged insulin fibres was obtained by modifying cation-exchanged PAN fibres with a solution of insulin in an acetate buffer under the following conditions:

insulin concentration of 0.25-2.0g/l, pH of 4-6, modulus of the bath 1:100 and 1:500, at 20±4$^{o}$C for 30-2400min. The pH value of the solution was regulated by the addition of acetic acid.

The concentration of chemotherapeutic agent in the solution during the modification has been determined by UV-spectrophotometry.

UV spectrums of the solutions of chemotherapeutic agents were recorded in the range of 200-400 nm. The concentration of procaine hydrochloride in the solution during modification was determined on the basis of maximum UV absorption at the wavelength of 291 nm. In the case of gentomicin sulphate, its concentration in the solution was determined on the basis of maximum UV absorption at the length of 248 nm, and concentration of insulin at the wavelength of 275 nm.

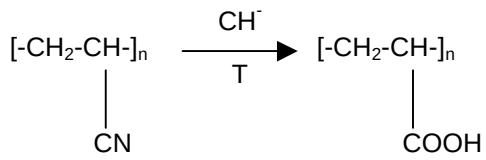
Investigation of the desorption kinetics of the chemotherapeutic agent from the biologically activated fibres. The selected chemotherapeutic agents with alkaline groups are fixed into cation-exchanged PAN fibres by chemical bonding, and are slowly released into the human organism over a definite period of time. The release mechanism involves the desorption and diffusion of chemotherapeutic agents. The desorption kinetics of the chemotherapeutic agent from the biologically activated fibres in distilled water and physiological solution was investigated under the following conditions: modulus of bath 1:200, at 20±4$^{o}$C, for 10min–24 hours. Preliminary experiments showed that the sorption temperature had no significant influence (when not exceeding 40$^{0}$C) on the amount of bonded chemotherapeutic agents. Bearing this in mind, as well as the simplicity of conducting experiments and the further application of these results, the temperature of 20$^{0}$C was selected as the sorption temperature. In order to compare the kinetics of sorption and desorption, the same temperature was also chosen for desorption. Our experiments of desorption in a physiological solution, at the temperature near to that of the human body, proved that there was no significant difference in the desorption rates.
The concentration of the chemotherapeutic agent in the solution during desorption was determined by UV-spectrophotometry.

The change of physiological salt concentration during desorption over time did not alter the results of spectrophotometric measurements, because all tested solutions were diluted with the physiological solution before spectrophotometric measurements. In order to continue the release of chemotherapeutics after the desorption was already under way, it is necessary to change the physiological solution, and we did such experiments, simulating an actual situation in the living organism's tissue.

## Results and Discussion

### Obtaining cation-exchanged PAN fibres

Attempts to immobilise antibacterial (gentamicin sulphate), anaesthetic (procaine hydrochloride) agents or insulin in PAN fibres by treating them with solutions containing the above-mentioned chemotherapeutic agents have not found practical application, as such agents were washed away too easily and quickly. Furthermore, fixing selected chemotherapeutic agents with proper functional groups of PAN fibres through chemical bonding also turned out to be difficult, due to the absence of such functional (acid) groups which could form a chemical bond with chemotherapeutic agents. In their structure, PAN fibres contain only a few carboxylic groups from itaconic acid which they contain. This accounts for the need to introduce essential functional groups into PAN fibres which can assimilate appropriate agents by chemical bonding. Carboxylic groups were introduced into PAN fibres by saponification of nitrile groups by aqueous sodium metasilicate solutions. The reaction can be illustrated as follows:




where n = degree of polymerisation. After modification treatment, the fibres were thoroughly washed with distilled water and dried at 50$^{o}$C. By changing the parameters of the saponification reaction

(concentration of $Na_2SiO_3$, modulus of the bath, reaction time and temperature), it is possible to obtain cation-exchanged PAN fibres with a different amount of carboxylic groups (0.13-3.5mmol per gram of fibres). The hydrogen atom present in carboxylic groups can be easily replaced with another cation, so the fibres can be used for effective isolation of different cations from solutions and gases, and as the basis for obtaining biologically activated fibres. Cation-exchanged PAN fibres allow chemotherapeutic agents with basic properties to bond chemically with the fibre. It is thus assumed that the addition of chemotherapeutic agent is based upon the reaction:

$$R_1\text{-}COOH + NH_2\text{-}R_2 \longrightarrow R_1\text{-}COO^-[H_3N^+\text{-}R_2]$$

in which: $R_1$ – the remainder of the polymer; $R_2$ – the remainder of the chemotherapeutic agent.

## Obtaining biologically activated fibres with antibacterial activity

Cation-exchanged PAN fibres contain carboxylic groups within their structure, which are thus able to combine with antibiotics with basic properties. Prior to the selection of antibiotics, scientific reports dealing with microorganisms' sensitivity to antibiotics were verified [6]. Gentamicin sulphate was selected because it is the most effective antibiotic in relation to the bacterial strains (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) which are considered representative of the hospital enviroment.

Cation-exchanged PAN fibres were modified with a water solution of gentamicin sulphate in different degrees of preparate concentration, lengths of modification, ion-exchange capacity (IEC) and form (H- and Na-form) of fibres. The amount of gentamicin sulphate into the fibres depends directly on the time of modification, the ion-exchange capacity and the form of fibres (results presented in Table 1), as well as on the concentration of gentamicin sulphate solutions. The maximum obtained amount of bonded gentamicin sulphate by cation-exchange PAN fibres is 140mg per gram of fibres. The obtained results are two times higher than the value found in literature [7], which proves that the method of saponification of nitrile groups by aqueous sodium metasilicate solutions is suitable for obtaining cation-exchange PAN fibres as the basis for obtaining biologically activated fibres with antibacterial activity; complicated methods of sulphonation or acrilic acid graft copolymerisation can thus be avoided.

The amount of bonded gentamicin sulphate is enough to develop desirable antibacterial activity in fibres due to the fact that Gram-negative bacteria strains (Escherichia coli and Pseudomonas aeruginosa) are sensitive to gentamicin with minimum inhibitory concentrations within a range of 0.06 to 8μg per ml. Among the Gram-positive organisms, most strains of Staphylococcus aureus are highly sensitive to gentamicin with minimum inhibitory concentrations within a range of 0.12 to 1μg per ml [6].

**Table 1:** The amount of bonding gentamicin sulphate by cation-exchange PAN fibres in mg of gentamicin sulphate per gram of fibres (modulus of the bath 1:400, modification temperature 20±4°C, antibiotics concentration of $7x10^{-3}$mol/dm$^3$)

| | **Time of modification, min** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Fibre ion-exchange capacity and form** | 1 | 5 | 10 | 15 | 30 | 45 | 60 | 120 | 60x24 |
| 2.20 mmol/g H-form | 54.3 | 80.5 | 81.0 | 86.7 | 86.7 | 86.7 | 86.7 | 86.7 | 86.7 |
| 2.20 mmol/g Na-form | 69.2 | 103.8 | 103.8 | 112.7 | 112.7 | 112.7 | 112.7 | 112.8 | 112.8 |
| 3.00 mmol/g H-form | 73.2 | 105.2 | 106.5 | 112.5 | 112.5 | 112.5 | 112.5 | 118.7 | 118.7 |
| 3.00 mmol/g Na-form | 77.9 | 117.0 | 117.7 | 127.6 | 138.6 | 138.6 | 138.6 | 138.6 | 138.6 |

In this way gentamicin sulphate is fixed into the fibre by the chemical bond, and it thus acts longer and more effectively. The desorption of bonded gentamicin sulphate has been studied *in vitro*, in the distilled water and physiological solution. Desorption of gentamicin sulphate in the distilled water did

not occur, which means that the biologically activated fibres with antibacterial activity possess high resistance towards wet treatments. Gentamicin sulphate bonded in biologically activated fibres was slowly released into the physiological solution. In the first day about 20% of the incorporated antibiotics were released.

## Obtaining biologically activated fibres with anaesthetic activity

Biologically activated fibres with anaesthetic activity used for local anaesthesia in the postoperative period can be obtained by the chemisorption of anaesthetic preparations into different ion-exchanged fibres. In this paper, cation-exchanged PAN fibres in H- and Na-form with different ion-exchange capacity were modified with a water solution of procaine hydrochloride during different periods of modification. A procaine hydrochloride concentration of $3.5x10^{-3}mol/dm^3$ was selected because, according to the literature [3], the recommended concentration of anaesthetic agent is 1.5 times the value of the ion-exchange capacity of the fibres. The obtained results, presented in Figure 1, show that cation-exchanged PAN fibres possess excellent sorption ability for procaine hydrochloride. As may be seen from the figure, the maximum amount of procaine hydrochloride is practically exhausted by the fibre during the first 90 minutes. The intensity of chemisorption depends on the form and ion-exchange capacity of PAN fibres. The maximum obtained amount of bonded procaine hydrochloride by cation-exchange PAN fibres is 182 mg per gram of fibres. The results obtained show that it is possible to obtain a biologically activated complex of ion-exchanged PAN fibre and anaesthetic agent which achieves a satisfactory anaesthetic effect (according to literature data [3], fibres with a local anaesthetic agent concentation of 5-15% show anaesthetic activity during 4-72 hours).

Desorption of bonded procaine hydrochloride has been studied *in vitro* in the distilled water and physiological solution. Desorption of procaine hydrochloride in the distilled water did not occur, which means that the procaine hydrochloride is bonded chemically and cannot be washed away. The procaine hydrochloride bonded in biologically activated fibres was slowly released into the physiological solution (Figure 2). In the first 2 hours about 8% of the incorporated anaesthetics were released. The results obtained indicate the possibility of obtaining an anaesthetic delivery system with controlled-release, and encourage further investigations into the behaviour of this system in the organisms of experimental animals.

## Obtaining biologically activated complex ion-exchanged fibres-insulin

Ion-exchanged PAN fibres, on account of the simultaneous presence of different functional groups (-COOH, -CN, $-CONH_2$), possess the ability of selective chemisorption of large organic molecules (insulin, adrenaline). They can therefore be used for the isolation and purification of these medical preparations, or for obtaining artificial storage of these preparations. Biologically activated complex ion-exchanged fibres-insulin were obtained by modifying cation-exchanged PAN fibres with a solution of insulin in an acetate buffer at different degrees of preparate concentration, pH value of solution, bath modulus, lengths of modification, ion-exchange capacity and form (H- and Na-form) of fibres.The results are presented in Figure 3. The results obtained show, together with an increase in concentation and bath modulus, that the absolute amount of bonded insulin increases while its relative amount decreases. The form of fibres (H- and Na-form) has a great influence on the amount of bonded insulin; fibres in Na-form are a more efficient sorbent. The amount of bonded insulin increases with an increase in lengths of modification and ion-exchange capacity of fibres. Changes in insulin chemisorption resulting from changes in pH can be explained by complete or partial ionisation of present functional groups into the fibres and insulin. The maximum amount of bonded insulin by cation-exchange PAN fibres of 800 mg per gram of fibres was obtained at pH 5 (near insulin's isoelectic point). Insulin bonded in biologically activated fibres was slowly released into the physiological solution at a rate of 1-1.5 mg per day and per 1g of fibres (the usual daily dose is 2mg). The behaviour of biologically activated complex fibres-insulin as an artificial store of insulin in the organisms of experimental animals (rats) with artificially provoked diabetes were investigated over a one-month period. Early *in vivo* studies in rats have given promising results, and additional work is under way. At this stage of study, the obtained biologically activated fibres with antibacterial activity and biologically activated complex fibres-insulin may be used together for better healing of diabetics' wounds.

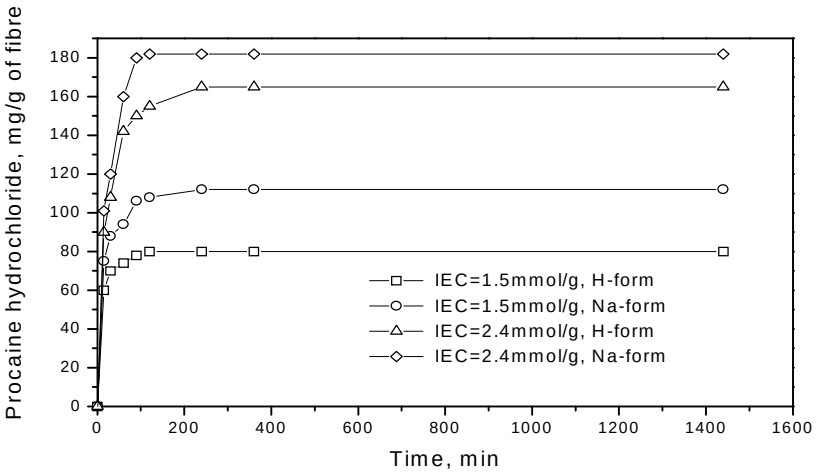



**Figure 1:** Kinetic curves of procaine hydrochloride chemisorption by cation-exchange PAN fibres (anaesthetic concentration - $3.5x10^{-3}$mol/dm$^3$, 20±4$^o$C bath modulus 1:200)

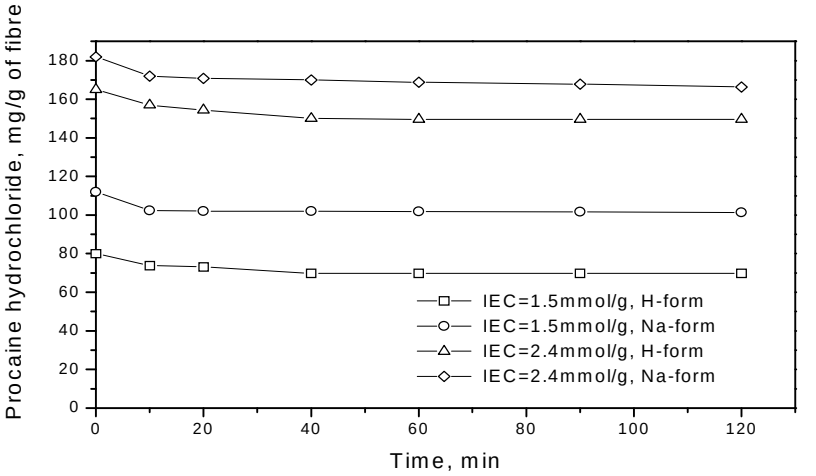



**Figure 2:** Kinetic curves of procaine hydrochloride desorption in physiological solution (temperature 20±4$^o$C, bath modulus 1:200)

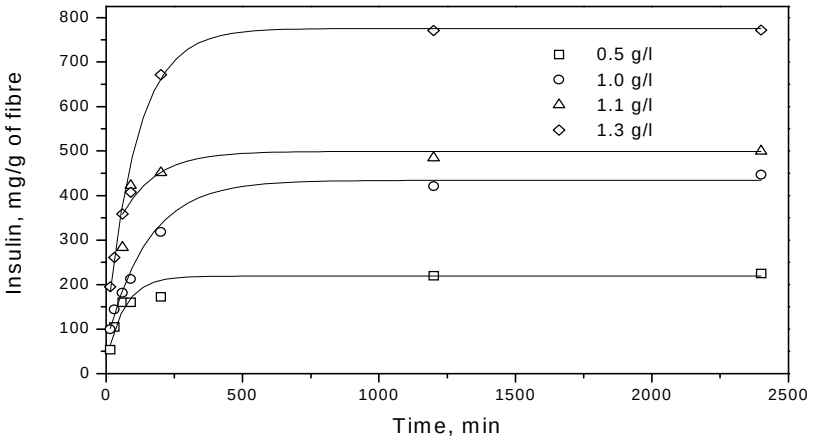



**Figure 3:** Kinetic curves of insulin chemisorption by cation-exchange PAN fibres depend on insulin concentration (IEC=1.7 mmol/g and Na-form of fibres, temperature 20±4$^o$C, pH 5, bath modulus 1:500)

## Conclusions

The results of the experiment show that PAN fibres must contain carboxylic groups in their structure in order to allow chemotherapeutic agents with basic groups to bond chemically with the fibre. An effective process for obtaining cation-exchanged PAN fibres by saponification of nitrile groups by aqueous sodium metasilicate solutions has been developed (large amount of carboxylic groups lead to good ion-exchange properties and short duration of modification).

Biologically activated fibres with antibacterial activity were obtained by modification of cation-exchanged PAN fibres with water solution of gentamicin sulphate. The maximum obtained amount of bonded gentamicin sulphate by cation-exchange PAN fibres of 140mg per gram of fibres is enough to develop desirable antibacterial activity in fibres. Modification of cation-exchanged PAN fibres with a water solution of procaine hydrochloride allows for the obtaining of biologically activated fibres with satisfactory anaesthetic activity (the maximum amount of 182 mg procaine hydrochloride per gram of fibres), which is maintained for quite a long time (up to 74 hours). Biologically activated complex ion-exchanged fibres-insulin, obtained by modifying cation-exchanged PAN fibres with a solution of insulin in an acetate buffer, contains up to 800 mg insulin per gram of fibres. with a usual daily dose of 2 mg, this amount is enough to control blood glucose level in diabetics for one year.

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