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Dedicated to the memory of
Professor Cristofor I. Simionescu (1920–2007

SYNTHESIS AND CHARACTERIZATION OF NOVEL FLAVONOID BASED BIOMATERIALS

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Acryloyl troxerutin (ATrox) monomer was obtained by the base-catalysed reaction of acryloyl chloride and troxerutin. Firstly, the ATrox was grafted onto polyamide knitted fabrics (PA) using ammonium persulfate as initiator. Secondly, the ATrox monomer was transformed into insoluble hydrogel by simultaneous radical polymerization and crosslinking. FTIR and SEM analysis of the troxerutin-grafted polyamide fibers confirmed the chemical bonding of ATrox molecules to polyamide. The kinetics of troxerutin release from troxerutin gels and troxerutin-grafted polyamide fabrics into phosphate buffer was examined. Troxerutin was slowly released as the results of the gradual splitting of esteric bond between drug and support.

INTRODUCTION

The use of textiles in medicine has a long tradition, starting with bandages and wound dressings. Nowadays, multifunctional textiles have been designed and produced for a controlled release of active species to different kinds of external wounds, in order to help and accelerate the healing process. Much progress has been obtained especially in the field of antimicrobial textiles, used for both prevention and healing purposes.[1]

Flavonoids are polyphenolic compounds found in many plants, vegetables, and flowers. They are valuable medicine and have been widely used clinically.

Venuroton and rutin are important flavonoid compounds and have been used for the treatment of venous stasis.[2] Venuroton, also known as troxerutin, (7,3',4'-tris[O-(2-hydroxyethyl)]rutin) (Trox) is an oral capillary presevatory drug, used for the relief of oedema and related symtoms in patients with chronic venous insufficiency.[3,4] Beside the oral application, troxerutin was intended for topical treatments as antioxidant,[5] or as protective agent on vein walls against the development of varicose veins.[6]

The combined treatment with bandages containing antibacterial or reparatory substances have offered substantial reduction of the healing time of venous ulcers.[7] The utilisation of textiles as drug delivery supports for the treatment of cutaneous diseases (*i.e.* venous ulcers of the inferior limbs) was not reported yet.

In this paper we developed a novel drug delivery support (biomaterial) containing chemically bound antiulcerous drug, namely troxerutin. A knitted polyamide material with polyurethane into structure is utilized as the support material in order to obtain bioactive dressing that provides durable infection-resistance and localized hemostatic properties.

EXPERIMENTAL

Materials

PA fabric (60% polyamide with 40% polyurethane (Elastan) was kindly supplied by Adesgo SA (Bucharest, Roumania)). Troxerutin was purchased from S.C. Antibiotice S.A. (Iaşi, Roumania). Acryloyl chloride was supplied by Fluka Co. (Buchs, Switzerland) and used without further purification. Ammonium persulfate (APS), N,N'-azobisisobutyronitrile (AIBN), triethylamine (TEA) and

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N,N'-dimethylformamide (DMF) were obtained from Fluka Co. (Buchs, Switzerland) and were of reagent grade.

Methods

Synthesis of Acryloyl troxerutin (Atrox)

In a typical synthesis, 1.12 g troxerutin (1.5 mmol) were solubilized in 8 mL DMF into a two necks round bottom flask. Then, 2.19 mL TEA (15.75 mmol) were added under stirring to the flask and the stirring was continued for five minutes at room temperature. Thereafter, 1.28 mL acryloyl chloride (15.75 mmol) were dropped by a funnel, under stirring at 0-5°C, on an ice bath. The reaction was allowed to continue for 1 hour at 0-5°C, and for 2 hours at 40°C. The product was purified by filtration and the filtrate was further precipited in diethyl ether. After extraction in chloroform/water, the organic phase was dried on anhydrous natrium sulfate, concentrated and dried under vacuum. The reaction yield was about 89%.

Preparation of troxerutin gel

Synthesis of troxerutin gel was carried out by simultaneous radical polymerization and crosslinking in DMF with AIBN as an initiator. Typically, 0.4 g of ATrox and 0.015 g AIBN were solubilized in 1.5 mL DMF. Dried nitrogen was bubbled through the solution for 30 min before reaction. The polymerization and crosslinking last 5 hours at 60°C. The obtained gel was washed succsesively with warm and cold distilled water, acetone and dried under vacuum at room temperature.

Preparation of troxerutin grafted PA

PA fabric (0.5 g) was washed for 30 min with a 2g/L nonionic detergent solution at 45°C and dried under air stream at room temperature. Then, the PA fabric was immersed in an aqueous APS solution (3%, w/v) for 20 min at room temperature. The sample was squeezed, thoroughly washed with water, and dewetted with a filter paper. The permanent grafting of ATrox on PA fabric was carried out by spraying and dipping methods. The first method consists in spraying on fabric samples of 1.5 mL aqueous solution of monomer (40%, w/v) containing three drops of non-ionic detergent as an emulsifier agent on the fabric surface (7 cm x 2.5 cm). The second method consists in immersion of fabric samples (11.5 cm x 3,5 cm) into a stoppered flask containing 5 mL of an aqueous solution of monomer (24%, w/v) and non-ionic detergent.

In both cases, the samples were maintained at 70°C for 2 h, thereafter washed with warm and cold distilled water, and dried for 24h under vacuum, at 40°C.

"In vitro" troxerutin release

In vitro drug release studies were performed by the batch method, using a phosphate buffered solution at pH=7.4 (NaH_2PO_4 20 mM and Na_2HPO_4 80 mM).[8] The grafted fabric containing troxerutin was used as such. The troxerutin gel was firstly dried under vacuum, and than ground into small particles (50-200 μm).

Typically, 100 mg of samples were placed into a flask containing 100 mL buffered solution at 37°C under magnetical stirring. Aliquots of the solution were withdrawn at given time intervals and the troxerutin content was spectrophotometrically determined at 350 nm, using a previously constructed calibration curve. The same volume of fresh receiving medium was added to replace the volume of the withdrawn samples.

Characterization

The FTIR spectra were recorded for ATrox, ungrafted and grafted fabric using a VERTEX 7 FT-IR spectrophotometer (Bruker, Austria).

SEM micrographs of untreated fabric and Atrox grafted fabric were taken by a TESLA BS 3001 type instrument.

Ultraviolet spectra for the determination of aqueous troxerutin concentrations were done with a UV-Vis spectrophotometer (Specord 200, Analytic Jena, Jena, Germany).

RESULTS AND DISCUSSION

Troxerutin-containing monomers were synthesized by the direct reaction of acryloyl chloride with troxerutin (Fig. 1).

The reaction between acryloyl chloride and troxerutin took place in DMF at moderate temperatures, in the presence of TEA as HCl acceptor. This reaction is ofen used for the synthesis of vinyl monomers[9-11] because of the mild conditions which allows acyl substitution of the hydroxyl groups without any damage of sensitive molecules.

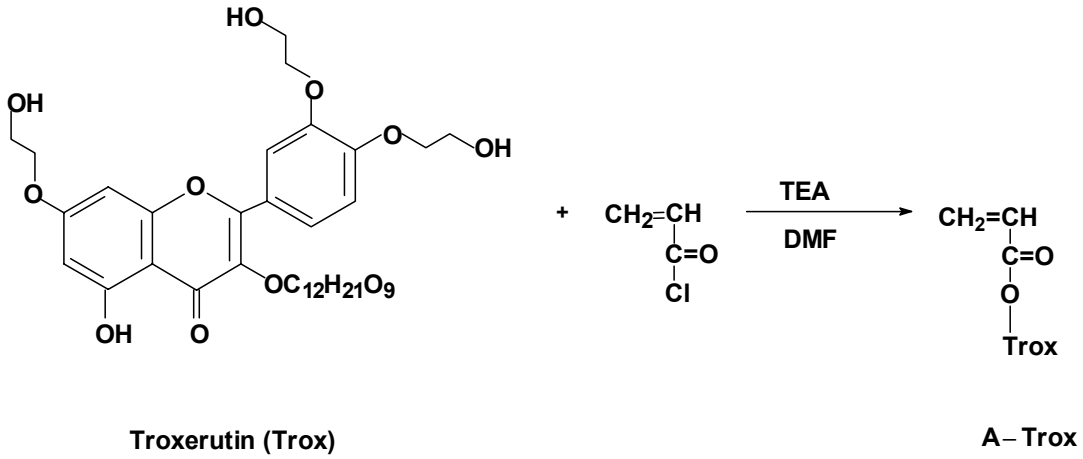

Fig. 1 – Chemical reaction of troxerutin with acryloyl chloride.

The introduction of double-bonds into troxerutin molecule was confirmed by thin layer chromatography (Rf 0.6 for troxerutin, and Rf 0.73 for ATrox, eluent n-butanol/etanol/water 5/4/3 (v/v/v)). Also, the occurrence of C=C vinyl stretching peaks at 980 and 947 cm^{-1}, and the absorbtion band of the ester carbonyl group at 1725 cm^{-1} in the infrared spectrum of the product (Fig. 2), confirmed the A-Trox synthesis.

The product of the esterification reaction was also characterized by the double bond content (DBC), expressed in meq./100g product. The DBC was estimated according to a method reported in literature.[12,13] A dried sample (0.2 g) was added to a stoppered flask containing 20 ml of $KBr/KBrO_3$ mixture, followed by addition of 10 ml H_2SO_4 (10%, w/v). The Br_2 released into the medium is able to undergo an addition reaction to the double bond of the acryloyl troxerutin sample. Back titration of the excess Br_2 was carried out using iodometric titration. The double bound content (DBC) was calculated using equation (1) and it was found to be 2.86 meq./g. (2.5 double bonds/ mol).

$$\text{DBC(meq./ g product)}=(V_B-V_S)\times 0.1/W \quad (1)$$

where W is weight of the sample (0.2 g); V_B is the volume of sodium thiosulfate (0.1 N) equivalent to the Br_2 released in the blank titration medium (ml); V_S is the volume of sodium thiosulfate used in the sample titration (ml).

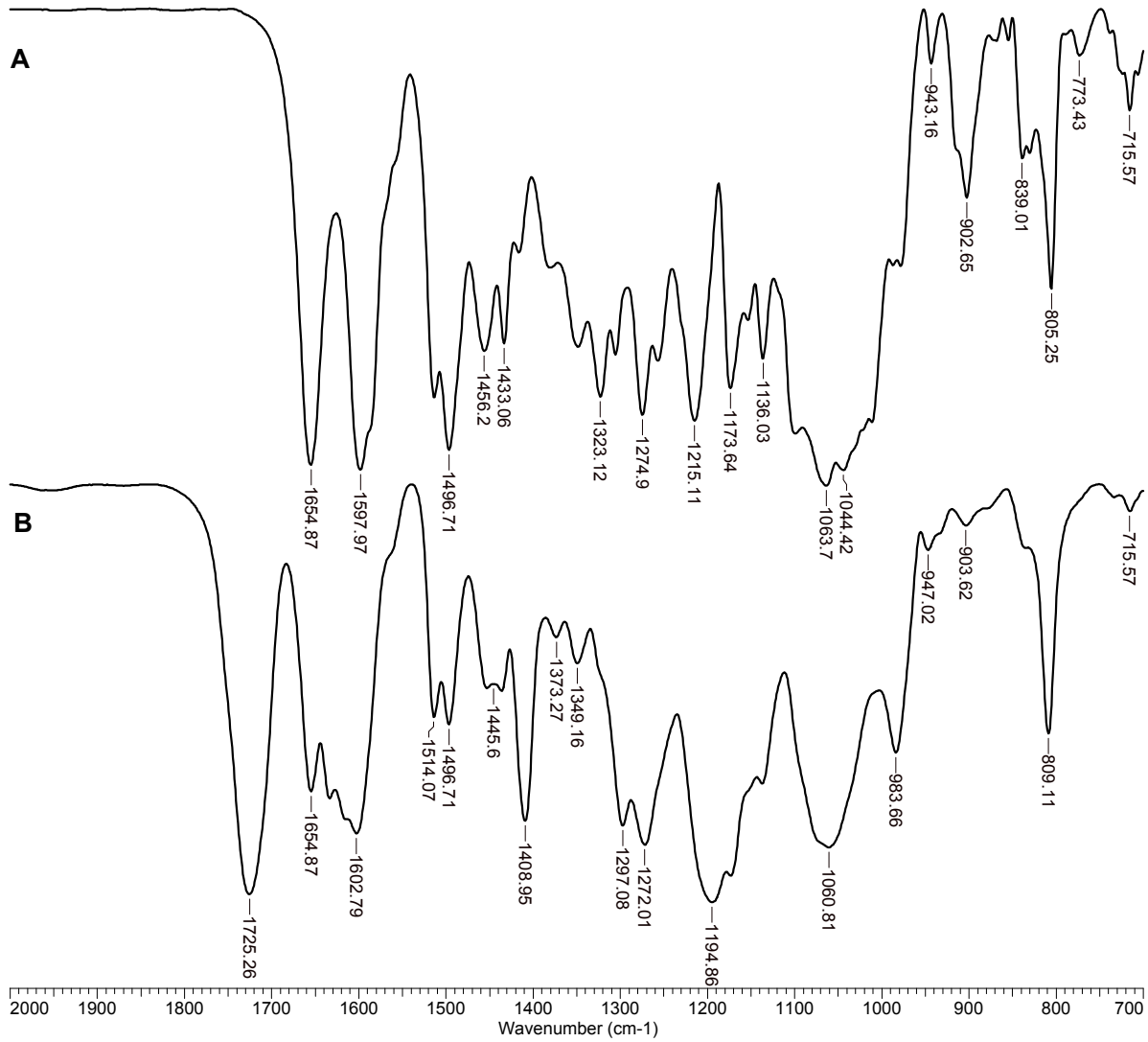

Fig. 2 – FT-IR Spectra of troxerutin (A) and ATrox (B).

Preparation of troxerutin gel was performed by the chemical crosslinking of the ATrox using ammonium persulfate as initiator (see experimental part).

The grafting of A-Trox onto PA fabric was performed for obtaining a PA fabric with troxerutin chemically fixed on it (Fig. 3).

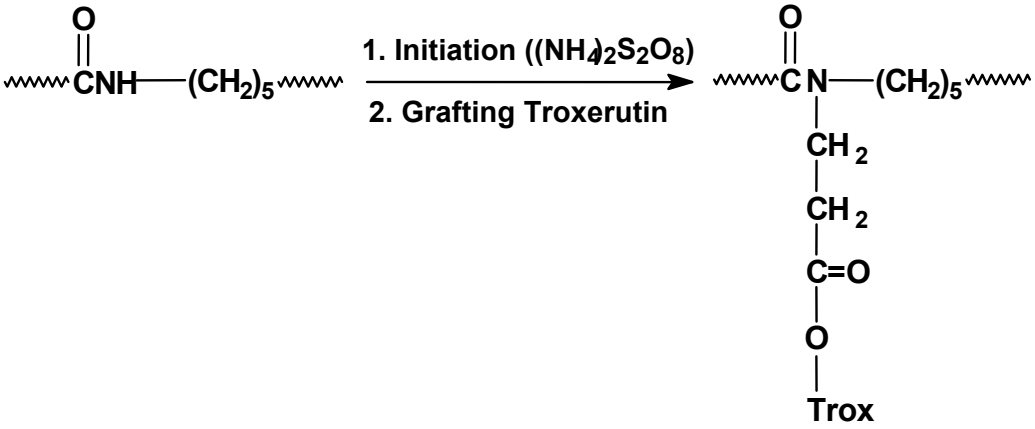

Fig. 3 – Grafting reaction of A-Trox onto PA fabric.

The amount of fixed troxerutin is dependent on the monomer concentration and grafting procedure used (Table 1). It was found that troxerutin grafting yield increased by using the immersion procedure (b), due to the wider surface of the PA fabric available for grafting (by spraying procedure, only one face of the PA fabric is covered with the monomer solution).

The IR spectra of the control PA and the grafted fabrics PA/ Trox have been investigated and are given for comparison in Fig. 4. The spectrum for PA shows absorbance bands that can be assigned to the amide group (1654-1542 cm^{-1}), and to NH_2 group (3303 cm^{-1}), N-H stretching at 3081 cm^{-1}, C-H stretching at 2928-2859 cm^{-1} (Fig. 4A). The grafting of troxerutin onto PA fabric is confirmed by the spectrum of PA/ Trox (Fig. 4B) that shows the additional absorbance bands of troxerutin (seen also in Fig. 2).

The SEM photographs of ungrafted and grafted PA fabric are shown in Fig. 5A,B. The grafting of troxerutin on the PA material seems to be in multilayers which covers more fibers (Fig. 5B).

Troxerutin release data analysis

The kinetic analysis of the release profiles was carried out according to the general equation:[14]

$$M_t / M_\infty = k\, t^n \qquad (2)$$

Where M_t is the cumulative amount of drug released at time t, M is the total amount of drug incorporated, k is the proportionality constant (the value of which depends on the structural and geometrical properties of the matrix) and n is the release exponent (its value depends on the mechanism of drug release).

As it can be seen from the curves presented in Fig. 6, the drug was faster released from the grafted PA/Trox than from the troxerutin gel. In fact, the release rate is determined by the splitting process of the esteric bonds in the release medium. In the grafted PA fabric the drug molecule is linked by a single esteric bond while in the gel one drug molecule might be linked by 1, 2 or more esteric bonds.

The amount of released drug is higher for the PA fabric grafted with drug by immersion procedure because the amount of linked drug itself is higher in this case. However, all these types of biomaterials are characterized by an initial phase of rapid drug release occurring over 5 hours, followed by a zero order release rate up to 5 days. The initial rapid drug release ("burst effect") might be due to the splitting of the esteric bonds located at the surface of all three types of biomaterials.

Table 1
Influence of grafting procedure on the amount of fixed troxerutin on PA fabric

Code sample	Grafting procedure*	Temp. (°C)	Monomer concentration (%, w/v)	Reaction time (hours)	Amount of fixed Troxerutin (%, w/w)
T #1	(a)	60	40	5	4.65
T #2	(b)	60	24	5	26.17

* First, the PA fabric was treated with the initiator solution for 30 min, at room temperature

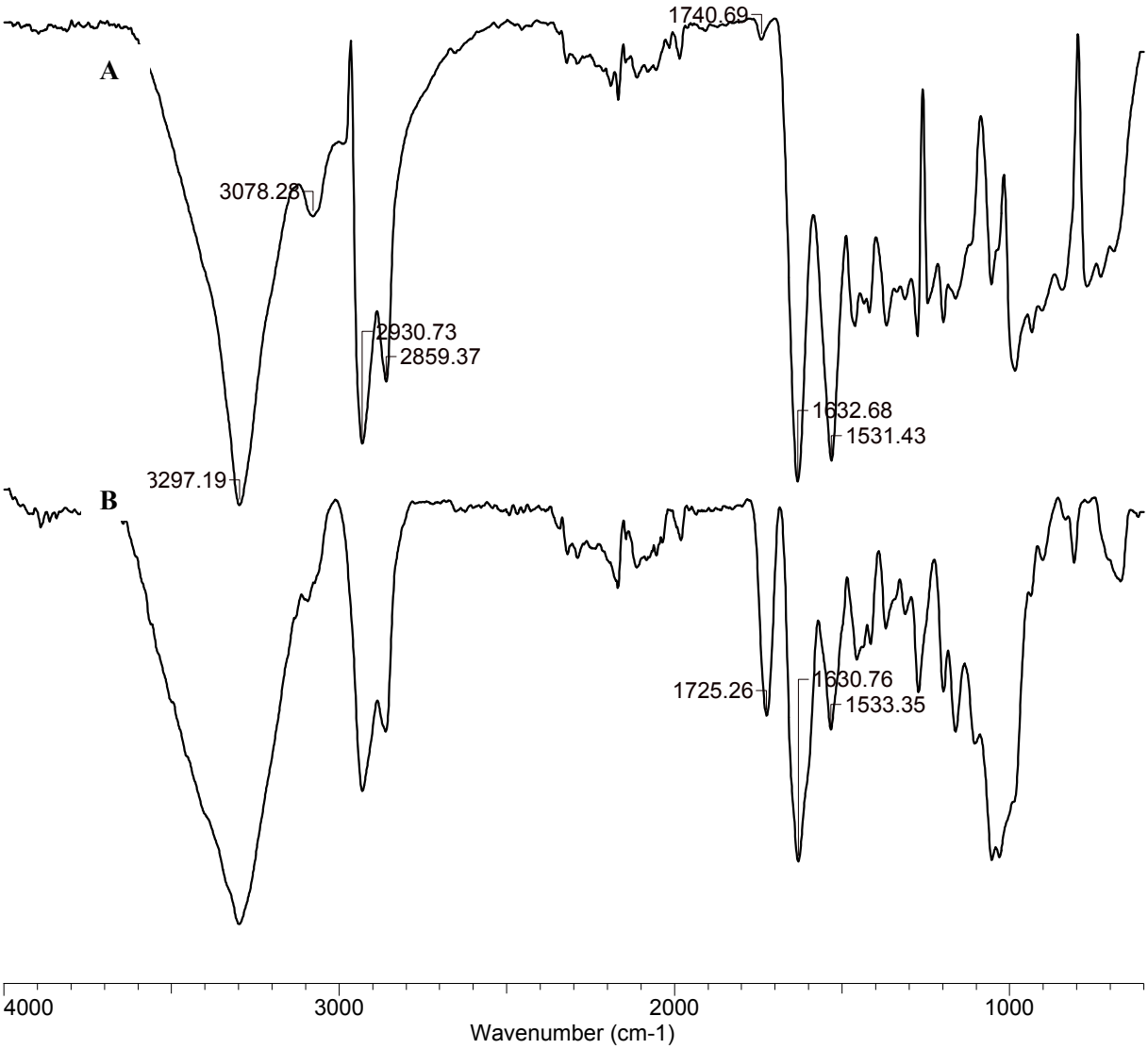

Fig. 4 – FT-IR spectra of original PA fabric (A) and grafted PA fabric (B).

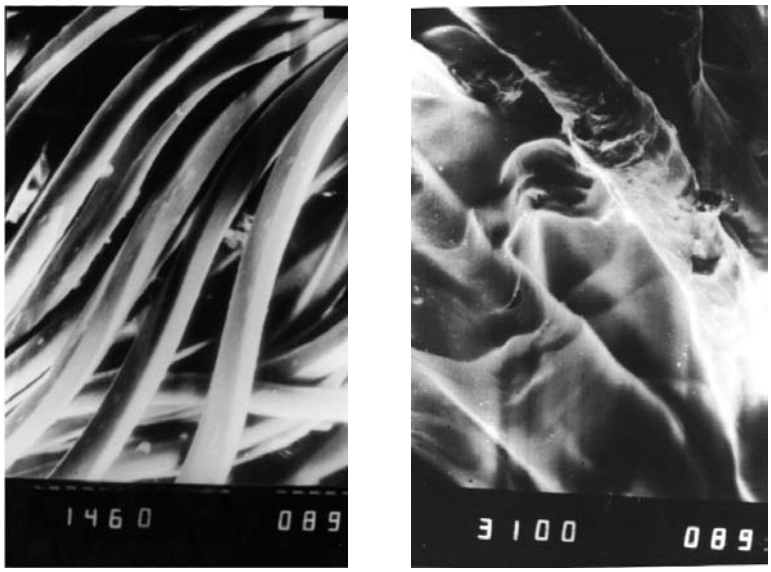

Fig. 5 – Scanning electron micrographs of untreated PA fabric (A) and PA/ Trox (B) (T#2).

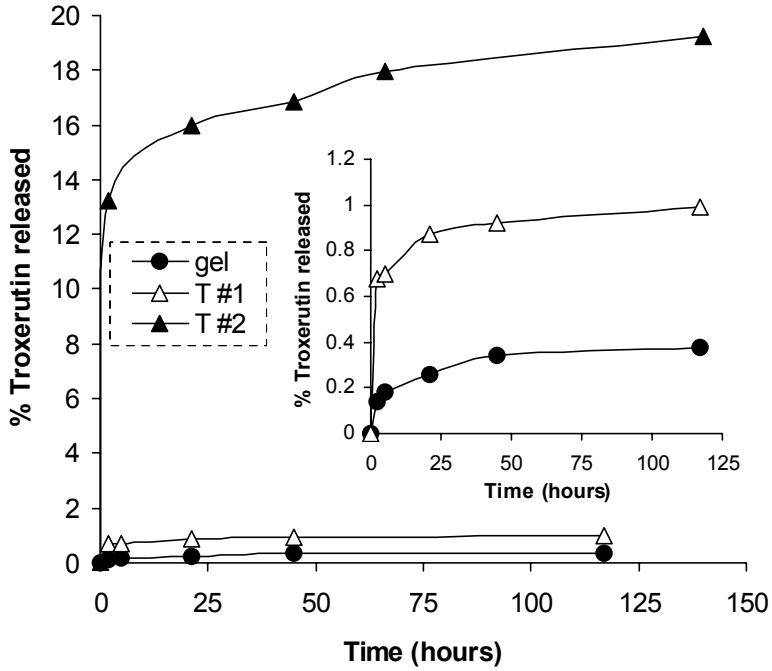

Fig. 6 – Release profiles of troxerutin in phosphate buffer (pH=7.4) from troxerutin gel (●), troxerutin grafted PA obtained by spraying (Δ) and immersion (▲) procedure.

CONCLUSIONS

Acryloyl troxerutin was successfully grafted onto polyamide fabric by two procedures: spraying and immersion. Also, insoluble troxerutin gels were prepared by simultaneous polymerization and crosslinking of acryloyl troxerutin monomer. FTIR spectral analysis provided the evidence of grafting of troxerutin onto polyamide fabric. SEM analysis confirm the presence of antiulcerous drug on knitted fabrics. Troxerutin was slowly released by the grafted fabric and by the troxerutin gel, indicating a possible clinical application.

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