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PHYSICAL PROPERTIES OF LASER IRRADIATED SCLEROSING FOAMS

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Abstract. The Polidocanol microfoam sclerotherapy is a potential treatment of venous disease. Recently, four years conducted clinical trials confirm that the Polidocanol microfoam injections assisted by pulsed Nd:YAG laser therapy improves the cure's efficacy. Started from these, laser beams interaction of liquid as well as microfoam Polidocanol was investigated. Results regarding the elements that may influence the foam stability of the sclerosing agent are discussed. The foam stability is increasing with the increment of sclerosant's concentration and with the increase of air percentage. The FTIR spectra suggest molecular structure changes of foam comparing with liquid polidocanol in both unirradiated/irradiated samples.

Key words: foam half time, FTIR spectroscopy, Nd:YAG laser, Polidocanol, sclerotherapy.

1. INTRODUCTION

The treatment of varicosities is very challenging because of the varying sizes, depths, flow patterns, and vessel thicknesses of leg veins. Amongst less invasive treatments, sclerotherapy assisted by laser therapy is a suitable approach especially for micro-veins, with caliber less than 1.5 mm [1].

The sclerotherapy is a minimally invasive percutaneous technique using chemical irritants to close unhealthy veins. A sclerosing substance is injected into the vein lumen, where produces a chemical irritation and endothelial inflammation. This process generates a local thrombus attached to the vessel's walls, which transforms it into a fibrotic cord [2]. Liquid but mostly foamed sclerosant drugs are employed in nowadays therapies. The most popular drugs worldwide used for the varicose veins foam sclerotherapy are detergent like sclerosants. The injection of foamed medicines has the benefit of maximizing the contact of the active agent with the vessel wall by displacing the intravascular blood. This leads to the minimum dilution and deactivation of drug by blood components. Also, less active substance concentration is needed for therapy. The use of foams allows better controlled drug delivery in order to obtain the wanted effect on the affected tissue.

Aqueous foams are dispersions of gas in liquid, stabilized by surfactant adsorbed at the air–liquid interface [3]. Aqueous foams are unstable systems that evolve over time by gravitational drainage of liquid, coarsening, and film rupture [4]. These features are dependent on properties such as foam wetness, the used surfactant, and bubbles sizes [3]. The efficacy of foam sclerotherapy ultimately depends on foam quality.

A large number of sclerosants are employed in the varicose veins treatment. One of the most used is Polidocanol (POL), a mild nonionic surface active substance (surfactant). Surfactants reduce the surface tension (ST) of liquids by dispersing among the surface molecules. As they possess a polar hydrophilic head and a non-polar hydrophobic tail, they are known as amphiphilic compounds [5].

Among the factors that influence the foam characteristics are: liquid to air ratio, active substance concentration, type of connector, syringe diameter, number of pump cycles, type of used surfactant and its concentration, rheological properties of the obtained solution, temperature etc. [6, 7]. The effects of adding different substances on the stability of POL foams by altering the ST and on the interfacial rheological properties of the fluids were also studied. It is shown that adding small concentrations of nonionic surfactants one may increase the foam stability with just a very small variation of the mean bubbles size [8].

There are several techniques that have been developed in order to generate foams for clinical use. The majority are obtained by cyclical mechanical agitation of the liquid substance in the presence of a gas, usually air (79% N_2 and 21% O_2), at gas/liquid ratios ranging from 1:1 to 8:1. One of the most used methods to generate foam is the Tessari technique, which uses a double syringe system bounded through a three ways connector [9].

Due to the availability of different procedures and materials, the POL microfoam sclerotherapy most likely achieves reasonable results. However, when varicose veins are very widespread in both legs, a high number of therapy and maintenance sessions are required [10–12].

Laser therapy is an alternative to microfoam sclerotherapy, particularly when there is a resistance to such treatment and in the cases of smaller veins calibers [13]. Dermatologic application of laser technology was initiated by Dr. Leon Goldman [14] and later some authors described the principle of selective photothermolysis [15, 16].

Different lasers have been used in the treatment of varicosities in the past decades, with variable results. However, studies comparing these interventions with microfoam sclerotherapy, which is regarded as the “gold standard”, conclude that only the Nd:YAG laser has shown similar effectiveness [17–19]. Nevertheless, limitation caused by the vessels caliber, deepness of penetration, pain at high fluences and the occurrence of relapses were reported [20, 21].

Fourteen years ago, at the Instituto Medico Laser (IML, Madrid, Spain), it was observed that applying the Nd:YAG laser tissue irradiation immediately after injection of POL microfoam, the venous lesions cleared almost completely, and the effects achieved appeared to be permanent. Several trials were performed so far in order to determine the optimal procedure, which showed that results were very good after a single session, and even better after a second follow-up session three weeks later [22]. The protocol was added to their daily practice as a recommended initial treatment for large areas of varicose veins.

With the aim to better understand the physical processes involved in the evolution of foaming POL but also the laser beam influence on this sclerotic agent, this study reports spectral investigations of both liquid and foam POL, including subsequent exposure to pulsed Nd:YAG laser radiation. There are also discussed results regarding the factors that may influence foam stability of POL, like liquid to air ratio and number of pumping cycles.

2. MATERIALS AND METHODS

POL or Laureth-9 (Sigma-Aldrich Chemie GmbH, Germany), belongs to the group of alkyl polyglycol ethers, commonly called alcohol ethoxylates. It is a nonionic surfactant whose chemical structure (Fig. 1) forms an alkyl chain of 12 carbon atoms and a chain of 9 ethylene oxide units [$C_{12}H_{25}O(CH_2CH_2O)_9H$] [23].

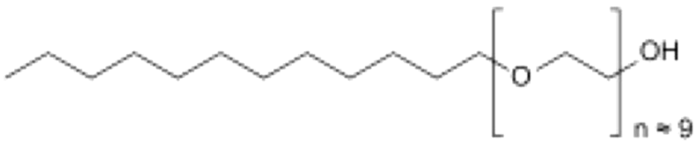


Fig. 1 – The chemical structure of POL ($C_{30}H_{62}O_{10}$, Lauromagrogol 400).

For spectral evaluation, as well as for laser beam exposure, solutions of POL 0.5% in a mixture of ultra pure water (u.p.w.) delivered via a sterile filter (TKA Pacific UP/UPW6, Thermo Electron LED GmbH, Germany), and ethanol (Merck Millipore, Germany). (1:1) were prepared.

The POL concentrations selected for foam stability evaluation are those mostly used in foam sclerotherapy and are commercially available at concentrations of 0.5%, 1%, and 3% in buffer solution (Kreussler, Germany).

The molecular weight of POL depends on the number of ethoxy units (E_n) in the molecule. For POL manufactured by Kreussler (E9), the molecular weight is approximately 600 [24].

Detergent like sclerosants may form mixed micelles with lipids, bind to hydrophobic portions of proteins or cause protein denaturation. Both anionic and non-ionic detergents can bind serum albumin at its hydrophobic regions. The interaction of surfactants with cell membranes and the eventual outcome is influenced by physical and chemical characteristics such as charge, critical micelle concentration (CMC) and aggregation number [25, 26]. POL, as a surfactant with a neutral large head group and longer hydrocarbon chains has milder properties; thus it solubilizes membrane proteins without affecting important structural features [27]. Its tendency is rather to form toroidal complexes than to solubilize membranes by extraction of phospholipid molecules directly from the membrane into preformed detergent micelles as the ionic detergents are doing [24].

The most used foaming procedure in clinical applications is the Tessari technique [24] and in our experiments it was employed the same method (Fig. 2).

a) b)

Fig. 2 – The Tessari foaming procedure [8] (a) and resulting foam consistency [2] (b).

One of the main factors that influence foam stability is the liquid drainage between bubbles under the influence of the gravity. The foam stability may be assessed based on the foam half time (FHT), which represents the time needed for half of the original volume of sclerosing solution to revert to liquid state [28]. In our experimental study FHT was measured along the scale of the syringe for quantitative imaging. Freshly generated foam in a syringe placed vertically with the piston set to the total volume of liquid/air mixture was employed in each survey session. Measurements follow the protocol described elsewhere [8]. Some studies found that the foam stability is affected by the type, size, and the rubber part of the disposable plastic syringe [24, 28, 29]. Our experiments employed two syringe sizes: one of 10 ml volume, with 15.7 mm in diameter, and the other one of 5 ml volume, with 12.2 mm diameter.

Foam stability dependence on the number of pump cycles, liquid/air ratio, and POL concentration was estimated. The physicochemical stability at gas/liquid interface of POL solution droplets was evaluated by dynamic ST measurements performed with a Drop Profile Analysis Tensiometer (PAT1, Sinterface, Germany).

Optical properties of POL solution were investigated by absorption spectroscopy using the UV/VIS/NIR spectrophotometer (Lambda 950, Perkin Elmer, USA). The used experimental spectral resolution was 0.05 nm for UV/VIS and 0.2 nm for NIR. Quartz cuvettes of 1 mm optical path were employed.

FTIR spectroscopy was also used for POL characterization. The FTIR spectrometer (Nicolet IS50, Thermo Scientific, USA) operates with Omnic 9 Standard software and the used experimental spectral resolution was 0.09 cm^{-1}.

A volume of 120 µL of POL solution 0.5% in u.p.w.-ethanol was exposed in successive sessions up to 30 min to 266 nm laser beam by utilizing the 4th harmonic (FHG) of the Nd:YAG laser (Excel Technology, model Surelite II, Continuum, USA) fundamental beam in the experimental arrangement schematically shown in Fig. 3.

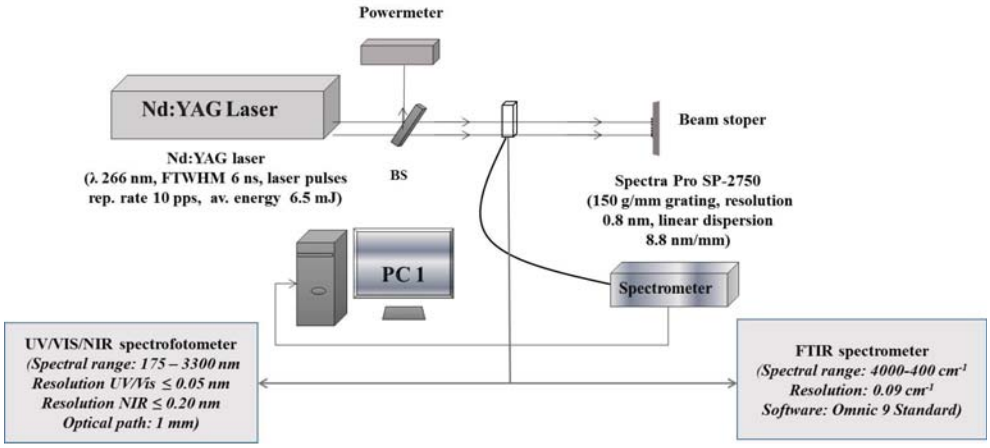


Fig. 3 – Laser exposure experimental set-up and the used survey devices.

The output laser beam was directed to the sample quartz cell of 1 mm optical path through an UG5 optical filter (not shown in Fig. 3) in order to cut-off the residual 532 nm radiation. 10% of emitted beam was split to a powermeter (QE25 Gentec, Canada) to monitor the beam energy on the sample cell. The laser radiation had the following characteristics: 266 nm – wavelength, 10 pps – pulse repetition rate, 6 ns – full time width at half maximum (FTWHM), and the beam energy on the sample – 6.5 mJ. After each exposure session, the effect of pulsed UV laser radiation on POL solution samples in bulk have been evaluated based on their FTIR absorption spectra.

3. RESULTS AND DISCUSSIONS

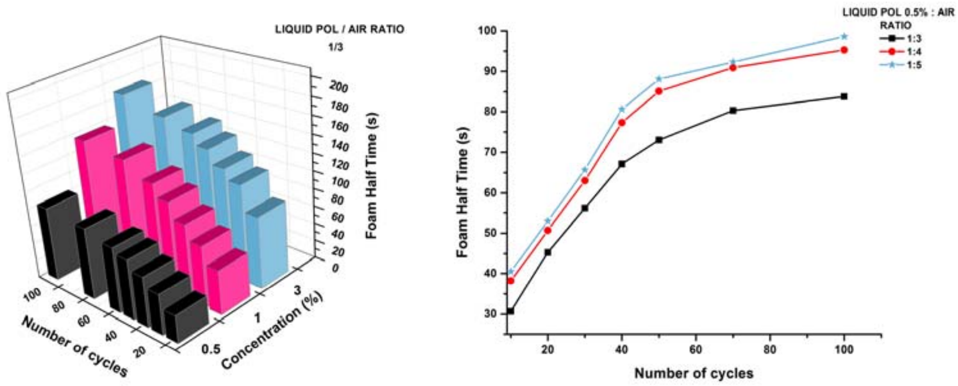
Considering the new implemented method to treat the varicose veins by POL microfoam sclerotherapy assisted by pulsed Nd:YAG laser therapy, three factors should be taken into account: * the drug sclerosing ability, * the possible increasing of the drug's potency under exposure to laser light, and * the influence of the laser beam on the surrounding tissues. Given the aim of this study, only the first two elements will be discussed.

Regarding the sclerosing skills of POL, clinical use attests the better results of microfoam sclerotherapy, as we mention in section 1 of this report. Although in the last couple of years foaming kits (Kreussler Pharma GmbH, Germany), and polidocanol endovenous microfoam, PEM (Varisolve®, BTG International, U.K.) are standardized and are commercially available [30], in clinical practice the most used foaming method remains the Tessari technique, due to its availability and facility. In this respect, the foam stability is the important issue, considering that it must assure the better intimate contact with the vein intern epithelium, and that it should gradually deplete for avoiding side effects [29].

Taking into account the factors that influence the foam stability, in our experiments the three most frequently used liquid/air ratios (1:3, 1:4, 1:5) have been selected. The foam was obtained through 10 to 100 pumping cycles.

The variation of foam stability in terms of foam half time regarding the above parameters was estimated. One can clearly observe that the foam stability raises by increasing the sclerosing agent's concentration (Fig. 4a) in the examined range.

For each measurement, after a number of pumping cycles (between 50 and 60), foam stability values attain a plateau (Fig. 4b) which indicates that bubbles sizes attained minimal values and they cannot break to smaller sizes despite the further dissipated energy for higher number of pumping cycles.



a) b)

Fig. 4 – Foam stability as function of the sclerotic agent (POL) concentration for 1:3 liquid/air ratio (a), and foam stability depending on the liquid/air rapport for a concentration of 05% POL (b) (on-line color).

The foam stability increases with the increment of air quantity but the difference between 1:4 and 1:5 is small. This is why we have selected 1:4 liquid/air ratio and 50 pump cycles as optimal conditions for all further experiments in this study. These results are partially illustrated in Fig. 4, considering that the same evolution is noted for all the experimented proportions and POL concentrations. The results obtained with the two types of syringes were close, with a small increase in stability for 10 ml syringe.

The physicochemical stability of gas/liquid POL films are evaluated by measuring the dynamic ST using a pendant droplet related technique. This analysis is based on the Young Laplace equation that uses the fitted profile of the droplet to calculate the value of ST [31]. In Table 1 the values of ST at equilibrium (after 4000 s) are presented for different POL concentrations, showing very close ST values for the investigated samples. The results suggest that the concentration is not a disrupting factor for the activity at the liquid/air interface of POL.

Table 1

ST values at the equilibrium for different concentration of liquid POL

Solution	ST [mN/m]
POL 0.5%	30.7 ± 0.3
POL 1%	30.5 ± 0.4
POL 3%	30.1 ± 0.3

The optical characterization of POL based on the absorption spectrum registered in UV/VIS/NIR spectral ranges highlights absorption peaks centered in NIR, as it can be seen in Fig. 5. The utilized solution totally transmits in UV/VIS spectral range. Previous studies provide similar results obtained for commercially available POL, features that are due to the superposed absorption properties of all the compounds included in the manufactured solution [32].

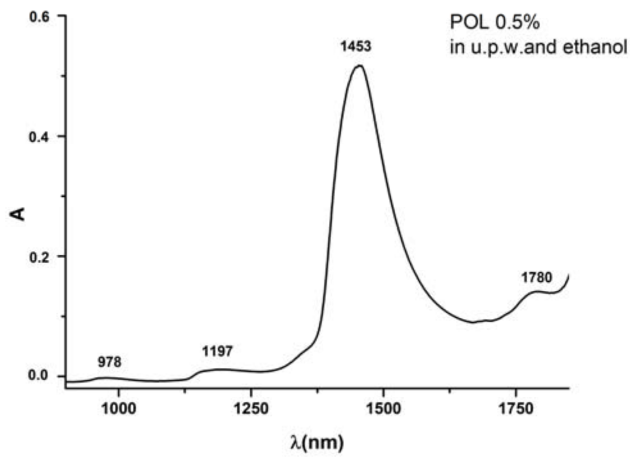


Fig. 5 – The absorption spectrum of POL solution in NIR spectral range.

Many reports prove that under controlled laser light exposure one may induce molecular structure changes of some drugs that could confer them enhanced or new pharmaceutical properties [33–39]. In this context, we exposed POL 0.5% solutions to pulsed Nd:YAG laser beams as described in the second section of this work. Following each irradiation session, FTIR spectra are recorded in order to highlight eventually structural modification.

Furthermore, POL microfoam was produced from the prior laser irradiated solution. Its optical characteristic was investigated by FTIR spectroscopy as well.

Even if the absorption of POL solution in UV is very low, we have exposed it to the FHG of the Nd:YAG laser. This was done taking into account previous reports outlining modifications of the absorption spectra between (250–285) nm following exposure to 1064 nm Nd:YAG laser beam, that are above the measuring error limits. This behavior was explained by a possible nonlinear absorption of 4 photons at 1064 nm, which would correspond to a transition at 266 nm [32].

The FTIR spectrum of unirradiated POL solution exhibits characteristic functional groups of the investigated molecule.

It is worth to note that C-H out-of-plane bending vibrations occur when POL is foamed. Also, C-O-H bending vibrations are affected in this case (Fig. 6).

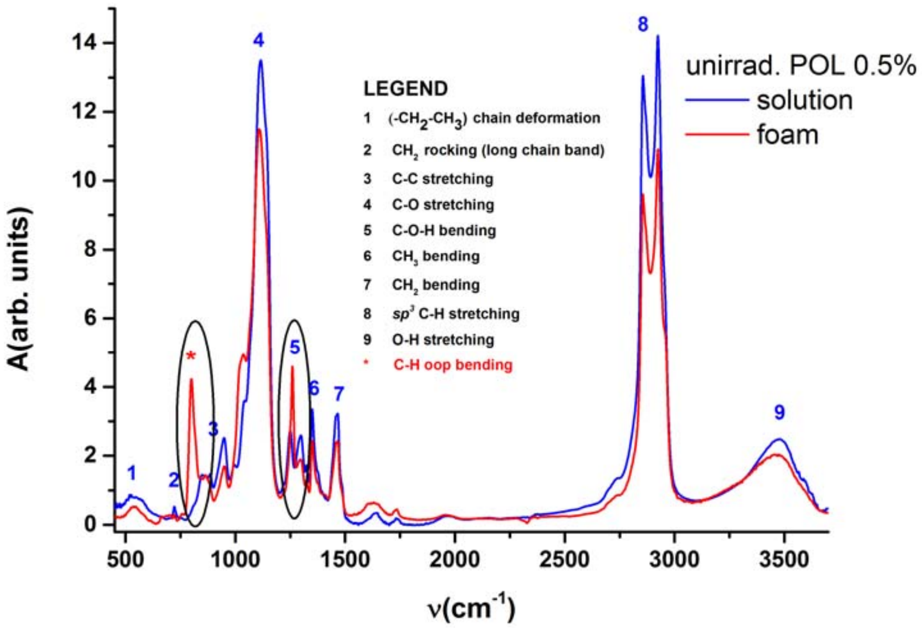


Fig. 6 – FTIR spectra of unirradiated POL (on-line color).

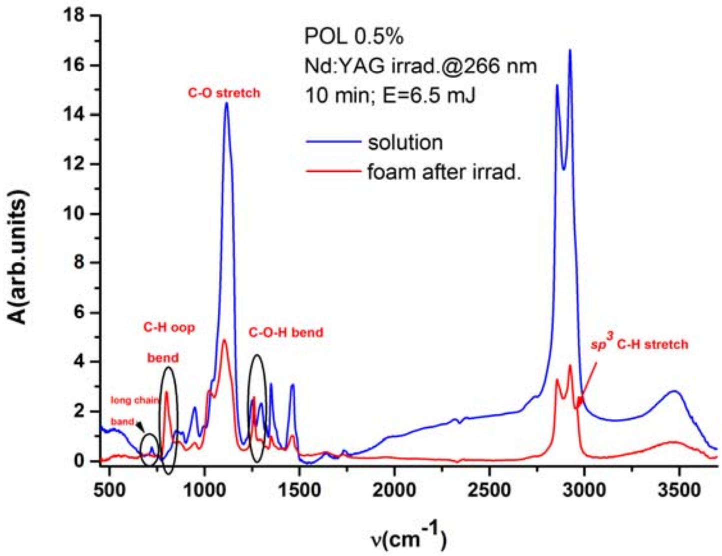


Fig. 7 – FTIR spectra of the 10 min exposed solution to pulsed Nd:YAG laser radiation (on-line color).

The FTIR spectrum of POL microfoam prepared after 10 min of laser beam exposure exhibits almost the same modifications that are highlighted for unirradiated samples, with bringing up the long chain bending and C-O stretching; sp3 C-H stretching vibrations are changed (Fig. 7). We have to mention that no important differences are registered when comparing the FTIR absorption features of unirradiated/irradiated POL solution, or the corresponding foam.

4. CONCLUSIONS

Results regarding the factors that may influence the foam stability of POL, like liquid to air ratio, and number of pumping cycles are reported. The physicochemical stability of gas/liquid POL films are evaluated based on the dynamic ST evaluation using a pendant droplet technique.

The foam stability is increasing with the increase of the sclerosing agent's concentration and with the increase of air percentage. This is why we have selected 1:4 liquid/air ratio and 50 pump cycles as optimal conditions for experiments in the study. These results confirm some experimental observations that are previously reported [40].

This study reports also spectral investigations of both liquid and POL foam, before and after pulsed Nd:YAG laser radiation exposure. The aim of these investigations consists in the better understanding of the physical processes involved in the evolution of foaming POL. It also regards the laser beam influence on this sclerotic agent.

By analyzing the FTIR spectra of both solution and POL microfoam in the two investigated circumstances (unirradiated and Nd:YAG laser exposed samples), molecular structure changes of foam comparing with liquid POL are found.

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