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DRAG REDUCTION IN NUTRIENT SALINE SOLUTION

Renata Trevelin da Silva
Edson Jose Soares
Universidade Federal do Espírito Santo, Departamento de Engenharia Mecânica, Avenida Fernando Ferrari, 514 Goiabeiras, 29075-910, Vitória, Espírito Santo, Brasil.
renata_trevelin@hotmail.com
edson.soares@ufes.br

Renato Nascimento Siqueira
Instituto Federal do Espírito Santo, Coordenadoria de Engenharia Mecânica, BR 101 norte, km 58, Litorâneo, 29932- 540, São Mateus, Espírito Santo, Brasil.
renatons.ifes@gmail.com

Leonardo dos Santos
Universidade Federal do Espírito Santo, Departamento de Ciências Fisiológicas, Avenida Marechal Campos, 1468 – Maruípe, 29.040-090, Vitória - ES, Brasil.
leodossantos@hotmail.com

Aloysio Abdo Silva Campos
Universidade Federal do Espírito Santo, Departamento de Ciências Fisiológicas, Avenida Marechal Campos, 1468 – Maruípe, 29.040-090, Vitória - ES, Brasil.
aloysiocampos96@hotmail.com

Abstract. *Drag reduction by high molecular weight polymers has been studied over the past decades and presents several applications in many industrial processes. Soluble drag reducing polymers, DRP, have also been shown to produce beneficial effects on blood circulation and may represent a way to treat cardiovascular disorders, like circulatory shock, atherosclerosis or hypertension. However, the efficiency of these polymers as friction factor reducers is not constant due to degradation caused by the turbulent flow. In the present work, we investigate the effects of concentration and degradation of three soluble polymers in the drag reduction capacity in a nutrient saline solution (NS) commonly used to maintain oxygenation and nutrition or organs in "ex vivo" studies. Polyacrylamide (PAM), Polyethylene Oxide (PEO) and Xanthan Gum (XG) are tested in a cylindrical double gap rheometer device for the concentrations of 10, 50 and 100ppm. For the conditions evaluated, the SNS composition does not change significantly PAM and PEO behaviour, showing that they can be satisfactorily diluted in this solvent without loss of efficiency as drag reducers. However, it modifies XG conformation, drastically reducing its efficiency. The experiments with tail arterial beds perfused with NS suggest that PAM is efficient to reduce the perfusion pressure.*

Keywords: *Bioengineering, rheology, drag reduction, polymer, nutrient saline solution*

1. INTRODUCTION

The friction factor reduction by addition of drag reducing polymers has been studied since Toms (1948) had reported this phenomenon. The author showed that the addition of small concentrations of polymers of long chains and high molecular weight reduce significantly the resistance to turbulent flow in tubes, what implies an increase in flow rate at constant pressure drop or a decrease in pressure drop at constant flow rate. Toms' effect has been intensively studied and has shown great success in many industrial processes like pipeline transportation (Burger and Chorn, 1980, Nijs, 1995), operation in oil wells, irrigation process (Singh et al., 1985) and firefighting system (Fabula, 1971, Figueredo and Sabadini, 2013).

It was also found that the reduction in hydrodynamic resistance is efficient in non-turbulent flow systems, such as pulsating flow (Driels and Ayyash, 1976). This find was extremely important to make the phenomenon useful in biomedical applications, enabling its use to reduce blood pressure and prevent cardiovascular disorders, such arteriosclerosis and hemolysis. The phenomenon itself is complex due to the interaction between the polymer long chains and the turbulence. In vascular flow applications, it becomes much more complex due to a non-permanent and pulsating flow, irregular velocity profile and possible interactions with tissue cells. One of the first studies in drag

reduction, DR, in vascular flow was made by Mostardi et al. (1976), which has observed a decrease in flow perturbation in aorta artery of dogs using Separan AP-30. Greene et al. (1970) has simulated a pulsating flow in a glass tube using polyacrylamide in calf blood and has obtained drag reduction. Experiments on preventing lethality after hemorrhagic shock were presented by Kameneva et al. (2004). Bessa et al. (2011) reproduced a pulsating flow in the tail arterial bed of rats using polyethylene glycol as drag reducing agent. Kameneva (2012) made a good review on "in vitro" and "in vivo" studies on drag reduction in blood circulation.

The biggest limitation of the use of drag reduction in industrial systems or biomedical treatments is the irreversible mechanic degradation of the polymer chains caused by the shear forces imposed by the turbulent flow. Researches have demonstrated that polymer degradation is dependent on concentration, molecular weight, Reynolds number, temperature and solvent properties (Soares et al., 2015). The use of PAM, PEO and XG with water as solvent has already been studied by many researchers, but their behaviour can change according to the solvent composition. The constituents present on the solvent can change the polymer chain conformation and affect DR. The influence of salt concentration on DR efficiency, for instance, has already been evaluated by some researchers (Kamel and Shah, 2009, Andrade et al., 2016), but other constituents present on the saline nutrient solution can also affect the DR efficiency.

Regarding the solvent for DR polymers, nutrient saline solutions are used as bathing medium for organs, tissues and cells "ex vivo", basically Krebs or Tyrode solutions, buffered with bicarbonate-carbon dioxide or organic buffers like HEPES, PIPES or TRIS. In any case, the purpose of these solutions is to preserve the isolated organ under a more physiologic condition, i.e., maintaining the osmolality, ionic strength, nutrition, oxygenation and pH at appropriate levels. Saline nutrient solutions are traditionally used in studies with post-hemorrhagic resuscitation (Jacob et al., 2006), biomechanical studies concerning the relationship between flow-pressure-resistance in vascular systems (Bergh et al., 2005), and "in vitro" studies on organ transplantation and recirculation regimen (Harris et al., 2015). Moreover, in vitro studies on the effects of drug/toxins (Rossoni et al., 2003), heavy metals (Fioresi et al., 2013) or vascular diseases (dos Santos et al., 2006; Tucci et al., 2007) in different isolated and perfused organs also are obligatorily conducted under perfusion with such buffered saline solutions to maintain oxygenation and nutrition of the tissue. However, these saline solvents can interact in a different way with the polymer, causing a distinct drag reduction behavior from the one observed in pure water.

The main goal of the present work is to investigate the efficiency of PAM, PEO and XG as drag reducing agents using a nutrient saline solution as solvent in order to analyze the influence of its components in the DR mechanisms. After that, perfusion tests were carried out in isolated tail arterial beds in order to evaluate the efficiency of PAM as drag reducer in conditions where the tissues can interact with the polymer and change the DR behavior.

2. EXPERIMENTAL APPARATUS AND PROCEDURE

In this work the rheometer tests were carried out to verify the efficiency of the polymers (PEO, PAM and XG) diluted in the nutrient saline solution. After that, perfusion tests were carried out in isolated tail arterial beds from Wistar rats in order to evaluate the efficiency of PAM as drag reducer.

2.1 Solutions preparation

The polymers PAM and PEO tested in this work have molecular weight of 5.0×10^6 g/mol, and XG, average molecular weight of 2.0×10^6 g/mol. Our chemical supplies were provided by Sigma–Aldrich.

Concerning the saline nutrient solutions, one of the most used NS for in vitro studies is Krebs-Henseleit, because it reproduces more accurately the physiology of the blood. Its composition is presented in Tab. 1. In this solution, the buffer is made with bicarbonate, which is bubbled with a carbogenic mixture (95% of O2 and 5% of CO2) in order to keep the pH constant at 7.4, i.e., the physiological pH.

Table 1 - Krebs-Henseleit composition.

Compound	Parts per Million
NaCl	6896
KCl	394
$MgSO_4 . 7H_2O$	236
KH_2PO_4	276
$CaCl_2 . 2H_2O$	182
$NaHCO_3$	1478
Na_2SO_4	167
Glucose	1970

In studies that aeration is not possible, Krebs-HEPES solution is usually used. It is based on the Krebs-Henseleit NS, modified by adding the synthetic pH buffer HEPES in the place of bicarbonates. Thus, pH could be maintained stable

without the need of bubbling (aeration) during the tests. Its composition is shown in Tab. 2. The two solutions (Krebs-Henseleit and Krebs-HEPES) are similar, differing by the component responsible for acting as buffer solution (component that maintains the pH stable). In the perfusion experiments, Krebs-Henseleit solutions are used, because it is more available and reproduces more accurately the physiology of the blood, but in the tests that aeration is not possible, the Krebs-HEPES solutions are normally used.

Table 2 - Krebs-HEPES composition.

Compound	Parts per Million
NaCl	5704
KCl	345
$MgSO_4 . 7H_2O$	292
KH_2PO_4	134
$CaCl_2 . 2H_2O$	275
$NaHCO_3$	2069
HEPES	4697
Glucose	1970

Rheometer tests were conducted using both distilled water (reference solution) and Krebs-HEPES (nutrient saline solution) as solvent in three different concentrations 10, 50 and 100 ppm of the polymers, which implies diluted solutions. To prepare the solutions, we first made concentrated solutions with 1000, 5000 and 10000 ppm, respectively, using distilled water as solvent. After 7 days, the concentrated solutions were diluted into this respective final concentration in distilled water and Krebs-HEPES and were stored in a refrigerator for 1 day prior to the completion of the experiments for complete dilution and homogenization and taken about 30 minutes before the test. This procedure was adopted to minimize the dilution time in order to allow, in future works, the use of blood to replace the nutrient solution using the same procedure, since the blood can coagulate or undergo microorganism proliferation. Krebs-HEPES' composition in shown in Tab. 1.

The saline nutrient solutions, Krebs-Henseleit, used in the perfusion experiments were prepared minutes before use, filtered and left for 30 minutes in the carbogenic bubbling bath to regulate the pH before starting the test. The polymer solutions were obtained from a 1000 ppm concentrated solution, which was stored for at least 3 days for dilution. Initially, a solution containing 100 ppm of the polymer was prepared and stored for 3 hours. After this time, part of the solution was stored and part of it was diluted to 50 ppm and 10 ppm by the addition of pure nutrient solution. These solutions still rested for about 2 hours to ensure total dilution before being placed in the thermal bath and bubbling.

2.2 Rheometer tests

The rheometer tests were carried out using a commercial rheometer Anton Paar Physica MCR 501. We obtained our results by using a double gap cylindrical geometry. The sample was located between the two rigidly interconnected coaxial and stationary surfaces, which have an axial symmetry. The rotor is a thin-walled coaxial tube located between these two fixed cylindrical surfaces, which can rotate over the sample holder's axis of symmetry at a given angular velocity. This geometry was used due to its large contact area, which provides measurements at small Reynolds numbers with good accuracy. The sample volume was 12.0 ml. Before all the tests, the sample rested into the test geometry over 1500 seconds to achieve the desired temperature, 37°C, which was chosen for being similar to the human body temperature.

The drag reduction, DR, obtained during the rheometer tests were calculated by Eq. (1):

$$DR = 1 - \frac{f}{f_0} \tag{1}$$

Where f and f_0 are the Fanning friction factor for the solution and the solvent, respectively, at the same Reynolds number, Re, defined by Eq. (2):

$$Re = \frac{\rho(\omega\bar{R})\bar{h}}{\eta} \tag{2}$$

Where η is the viscosity, ρ is the specific mass of the solution, $\omega\bar{R}$ is the characteristic velocity, in which ω is the angular velocity of the rotor and $\bar{R}$ is the average radius, and $\bar{h}$ is the average spacing between the walls of the cup and the rotor (see Silva, 2017).

The friction factor, f, is calculated by Eq. (3), based on the shear stress, τ, specific mass of the solution, ρ, and characteristic velocity, $\omega\bar{R}$.

$$f = \frac{2\tau}{\rho(\omega\bar{R})^2} \tag{3}$$

Viscosity and flow curves were obtained for all concentrations in order to understand the rheological characterization of the fluid. These tests were performed gradually increasing the rotational speed from 0 to 3000 rpm, which is the maximum speed of our equipment, the Fanning friction factor was displayed as a function of Reynolds number, and it was measured at 600 points over 600 seconds, sufficient time to develop the turbulent structures.

Constant shear rate tests were performed to analyze the loss of efficiency along the time of the experiments. The tests with a constant rotation speed were used to display drag reduction as function of time and it extended over 3600 seconds. 7200 points in logarithmic scale were measured.

2.3 Perfusion tests

The perfusion tests were carried out with isolated perfused tail arterial beds from Wistar albino adult male rats. All animal manipulation was carried out in accordance with the institutional ethics committee on animal use (Protocol 18/2016 - CEUA / UFES). The tests aim to evaluate the effect of the NS polymers concentration on drag reduction (DR) during the perfusion experiment.

The experimental bench consists basically of: a thermostatic bath (1), responsible for maintaining the temperature of the solution to be perfused and the tail arterial bed at 37 0C; a peristaltic pump (2), responsible for maintaining pulsatile flow; a bubble collector, used to prevent any bubbles or air entering the system from reaching the artery; a pressure transducer (3), used to measure the perfusion pressure of the organ (4); and a microcomputer (5) equipped with a data logger system (Biopac Systems, model MP35), responsible for receiving and storing the pressure transducer information. Figure 1 shows the simplified scheme of the perfusion system.

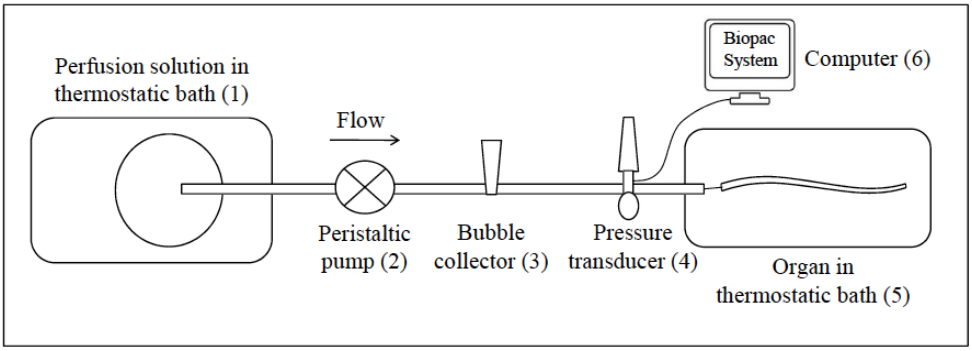

Figure 1. Perfusion system schematic drawing.

The perfusion technique of the tail arterial bed was similar to that used by Bessa et al. (2011). The tail artery was perfused with nutrient solution at a constant flow rate of 2.5 mL/min, maintained for about 30 min for stabilization (this is the basal flow rate, i.e., the blood flow rate in the organ when the animal is alive and in normal condition). After the stabilization time, some procedures were performed to test the endothelial integrity. It was considered that the endothelium remained intact when the relaxation percentage was greater than or equal to 30%.

After the protocol used to test the integrity of the endothelium, the effect of the polymer solutions in the tail arterial bed was evaluated. Initially, the flow-pressure relationship for the pure Krebs-Henseleit nutrient solution, without addition of any polymer, was obtained. The flow was maintained for 3 minutes in each flow rate: 1.25, 2.5, 5, 7.5 and 10 mL/min. The flow rate was altered by increasing the peristaltic pump rotation. After completion of this protocol, the basal flow rate (2.5 mL/min) was returned and maintained for about 7 minutes for stabilization. After this time, the solution was changed for another, containing 10 ppm of polymer, and 8 minutes was waited to ensure that all previous solution has passed through the system. The same process was repeated to obtain three subsequent flow-pressure relationships with concentrations of 50 ppm and 100 ppm of polymer solutions.

Once the artery diameter is not constant, Reynolds number and friction factor are not constant either. In order to quantify the drag reduction phenomenon, DR was defined in Eq. (4) as a function of the pressures involved in the system, according to Savings (1964). The author used the term drag ratio (D_R) to represent the relationship between the pressure drop for the drag reducing polymer solution (ΔP) and the pressure drop for the solvent (ΔP_0) in the same flow rate and pipe length.

$$DR = (1 - D_R) = 1 - \frac{\Delta P}{\Delta P_0} \tag{4}$$

This way, a solution can be said to be drag reducer if its drag ratio (D_R) is positive and less than 1.

3. RESULTS AND DISCUSSION

At first, the tests performed in the rheometer with the Krebs-HEPES solutions will be presented and discussed in order to evaluate the capability of the polymers to reduce drag when diluted in the saline nutrient solution (NS). In a second stage, the perfusion tests results, with PAM and the Krebs-Henseleit NS, will be shown and the performance analyzed.

3.1 Rheometer tests

In Figure 2 we show the Fanning friction factor in Prandtl-von Kármán coordinates for three different concentrations of the polymers, diluted in distilled water, DW, and Krebs-HEPES nutrient saline solution, NS. The rotation speed was gradually increased from 0 to 3000 rpm over 600 seconds. As widely reported by a number of researchers, the friction factor falls with increasing concentration. It is clear that the curves at different concentrations become far from each other with increasing Reynolds number, which implies that the slopes of the curves are an increasing function of concentration, as reported by Pereira et al. (2013).

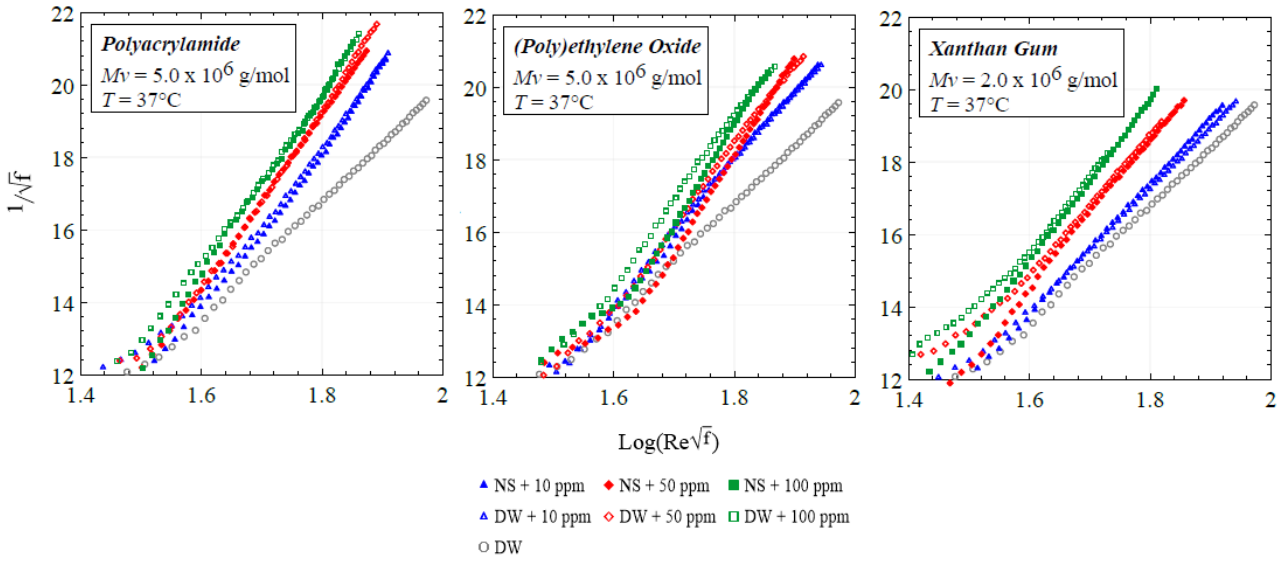

Figure 2. Fanning friction factor, f, in Prandtl-von Kármán coordinates, as function of Reynolds number, Re.

Figure 3 shows the effect of the concentration of the studied polymers on drag reduction with time to take in account the loss of efficiency of the polymers as drag reducers. The tests were carried out in the fix Reynolds number of 1100, where the rotation speed was kept constant over the 3600 seconds of measurement. As expected, the drag reduction efficiency increases by increasing the solution concentration. A maximum level of drag reduction is sustained during a period and after that time the molecular scission became more pronounced and the efficiency of the polymer decreased until it reaches its asymptotic value, when the degradation of the polymer chains stops and molecular weight achieves a steady state. In our tests, the asymptotic value is not so clear, mainly for the concentration of 10ppm, because after 3600 seconds the evaporation of the sample became very pronounced, making the measurements not so clear and accurate.

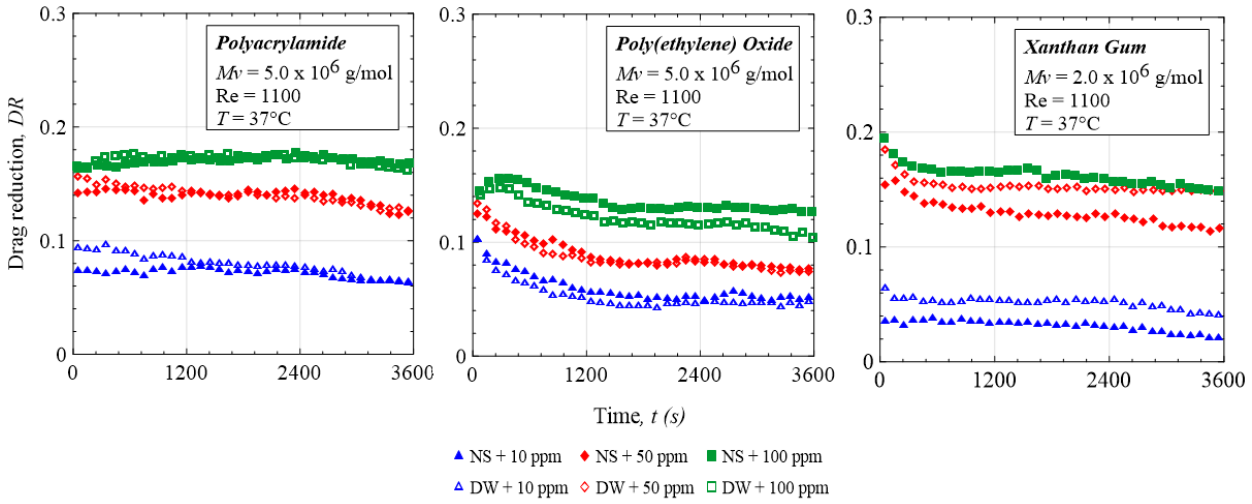

Figure 3. Effect of concentration on DR as function of time.

We can see in Fig. 3 that the drag reduction decay over the time and this loss of efficiency is more pronounced for the lower concentrations of polymer, what suggests that high concentrated solutions are more resistant to the degradation imposed by the flow.

Here our main interest is to highlight the differences between solutions in distilled water and the nutrient saline solution. It seems that the composition of the nutrient solution does not affect the phenomenon of drag reduction in PAM and PEO solutions, but affects XG solutions' efficiency, making XG solutions less efficient as drag reduction agent. Tests for XG solutions with 100 ppm concentration in distilled water were not possible to be done, because of equipment limitations. As shown in Fig. 2 and Fig. 3, the results obtained for both solvents presents quite the same behaviour for PAM and PEO solutions, therefore, PAM and PEO seems to be appropriate polymers in nutrient solutions for drag reducing purposes. Although, for the cases that were evaluated, XG does not seems to be appropriate for uses in nutrient saline solutions.

3.2 Perfusion tests

The second stage of this work aimed to evaluate the DR performance of the polymers in the NS when applied to tail arterial beds of rats. As the tissues were kept alive, it was possible to evaluate the influence of cell interactions on the drag reduction promoted by the polymers. The tests were carried on five biological replicates (each animal is used in only one replicate) but only the mean values are presented with the respective standard deviation. The standard deviation bars were plotted in only one direction to make it easier to see the results. Fig. 4 shows the results of mean tail artery perfusion pressure as a function of flow rate for PAM solutions.

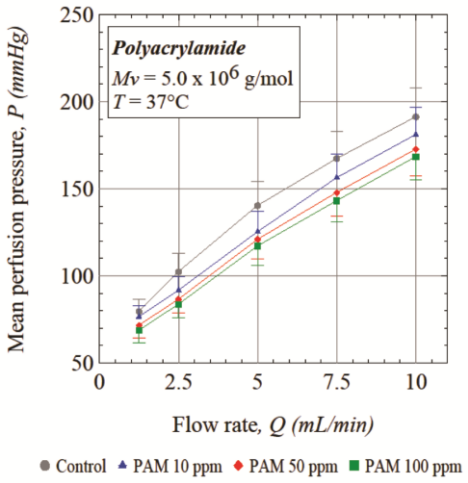

Figure 4. Perfusion pressure as function of flow rate

The use of PAM reduces perfusion pressure and the higher the polymer concentration the lower the perfusion pressure observed. For the basal flow rate (2.5 mL/min), for example, the perfusion pressure for the pure nutrient solution (control) was 102 mmHg, dropping to 91.7 mmHg when 10 ppm of PAM was added to the perfusion fluid and to 83.7 mmHg when 100 ppm of PAM was used. The results observed are in agreement with the obtained by Greene et al. (1980), who also observed drag reduction by PAM in their *in vitro* tests with pulsatile flow in tubes using calf blood.

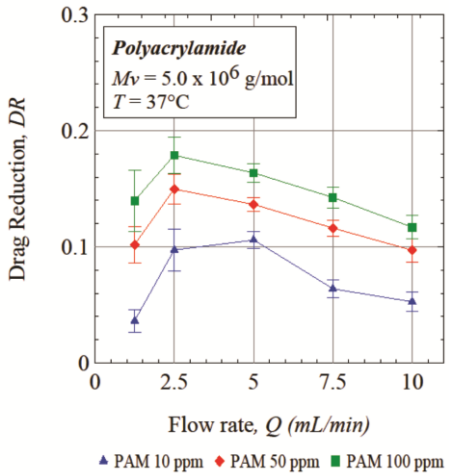

Figure 5. Drag reduction as function of flow rate

Concerning drag reduction, the results are displayed on Fig. 5. Since it was not possible to measure the diameter of all arteries and it changes with the flow, it was not possible to obtain Re and determine the friction coefficient, therefore, the drag reduction presented here follows Eq. (4). According to Fig. 5, the drag reduction increases with PAM concentration. For a given concentration, DR initially increases with the flow rate, remains constant for a while and begins to drop after that, which is observed for the three concentrations. If we were analyzing the drag reduction in a tube, this result would seem strange, since it is known that DR increases with the increase of Re but a reasonable physical explanation is the increased degradation of the solution as it passes through the pump when the flow rate is increased. Besides, in this case, we must take into account not only hydrodynamics but also physiological mechanisms.

4. CONCLUSIONS

Experiments were carried out in a rheometer with a double-gap rotary geometry in order to analyze the efficiency of the polymers PAM, PEO and XG as drag reducers in a saline nutrient solution (NS) when compared to efficiency in distilled water (DW). The results in the rheometer show that PAM and PEO do not have their efficiency greatly altered when diluted in the NS, but XG was more sensitive to the salts and sugars present in the nutrient solution. Thus, the Krebs-HEPES solution seems to be a good solvent for PAM and PEO and a poor solvent for XG. The experiments performed in a tail arterial bed of normotensive rats with pulsatile flow, in order to evaluate the efficiency of PAM when submitted to the flow in an organ subject to the interactions with the tissues show that PAM is a good drag reducer, with the perfusion pressure drop being noticeable with increasing solution concentration.

5. ACKNOWLEDGMENT

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