Plastified polyvinyl chloride for antimicrobial medical device applications

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Biocompatible synthetic polymers play an important role in medical applications, such as prosthetic devices (drains, catheters, hip, knees, etc.) employed at the present time by millions of patients. Polymers used as prosthetic devices include polyurethanes, silicone rubbers, teflon and vinyl polymers or copolymers. The possibility of utilization of polyvinyl chloride (PVC) material in prosthetic medical devices depends on biocompatibility, chemical and thermal stability, and suitable mechanical properties. Since PVC is largely used in medical tubing for transport of biological fluids, low amounts of water adsorption and wettability are very important for the production of catheters, implants, etc., in order to avoid biofilm formation on the surface. We present the development of medical grade plasticized recipes based on PVC with applicability in devices type catheters with antimicrobial properties and the comparison of surface analysis, mechanical and biocompatibility properties of PVC samples with antimicrobial activity, those flexibility is provided by different plasticizers.

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1. Introduction

In the medical industry the medical device sector is a domain dynamic and growing, that involved important efforts performance for imposing new material to support the development of new products. Manufacturers of medical devices for single use, are constantly looking for new products and new materials to expand their capabilities.

Tubular medical devices type catheters, sounds or drains are commonly used in hospitals to remove fluids from the body or to administer a drug. The nature and chemical composition of the biomaterials tubing is different, many of them contain plasticizers and additives that improve the physical and chemical properties and biocompatibility. The biocompatibility of these devices depends on the material, its surface and the body's response in contact with the device. The use of urinary catheters type medical devices in the long term, may cause adverse effects to human body due to alterations in the properties of the polymeric material, the loss of plasticizer (after several days in contact with blood or tissues, shows loss of about 40 - 60% from the initial quantity in the recipe), surface modification and adhesion of mineral salts. As a result, there may appear allergies [1-2]. The mechanism by which infection develops is not known, but the phenomenon of bacteria adhesion phlebitis, debossing, infections, and deflation to the polymer substrate is a critical factor in initiating colonization and subsequent infection. The adhesion of different microorganisms on the catheter surfaces and their survival depends on the interaction of the surface of the biomaterial [3].

PVC is substantially an amorphous material and it is never processed as only polymer, so, is necessary its formulation by incorporating several additives. Such as, typical recipe contains PVC resin, primary and secondary plasticizers, heat stabilizers, internal lubricant, processing aids, impact modifiers, fillers, pigment, UV stabilizer in order to make it processible. Because PVC is a relatively rigid and brittle polymer; flexibility is achieved through the addition of chemical plasticizers [4]. Various plasticizers are used to accomplish the flexibility, among them is DEHP [Di-(2-EthylHexyl) Phthalate] is the most frequently used in PVC compounds for medical devices manufacture. DEHP may migrate from the device to the human body, resulting in a certain degree of patient exposure. The effect of release plasticizers in the human body can cause cytotoxic phenomenon, inflammation of tissues, and infection [5 – 7].

This paper present the preparation of medical recipes based on plasticized poly(vinyl chloride) (PVC) as control recipes and antimicrobial recipes. It were performed three types of PVC recipes plasticized with addipate (P1), citrate (P2) and DEHP (P3). Content of plasticizers were 30% and 1.6% soy bean oil as secondary plasticizer. Recipes were loaded with antimicrobial additives based on silver salts nanoparticle 10%wt, so as content of plasticizers remain the same that in recipes control, but the amount of PVC was lower. Compounding of PVC recipes were performed

in usual conditions similar to industrial processing of plasticized PVC.

The purpose of this study it was to prepare several antimicrobial recipes based on polyvinyl chloride, and to investigate the qualitative effect of two ecological bio-based plasticizers from citrates and addipates category in comparison with DEHP, on the mechanical properties of the PVC compounds in order to replace DEHP. The results will represent a guideline to improve medical recipes to achieve sensitive and safe applications, which provide an excellent compatibility and flexibility at low temperatures, and optimizing the processing condition.

2. Experimental details

2.1 Materials

Materials were purchased as follows: polyvinyl chloride with K-vert 70 from Ongrovil-Hungary, plasticizers: di-2-etilhexyl phthalate (DEHP) from Sigma-Aldrich, tributyl 2-acetylcitrate (sort Proviplast 2624) and bis(2-(2-butoxyethoxy)ethyl) adipate (sort Proviplast 01422) from PROVIRON Belgium, soy bean oil from Monsanto Company, calcium stearate and zinc stearate from Baerlocher Additives, Irganox from BASF, and silver salts nanoparticles. PVC and plasticizers characteristics are presented in the next tables.

Table 1. Characterisctics of PVC Ongrovil

Properties	*DOP*	*Proviplast 01422*	*Proviplast 2624*	*Soy bean oil*
Density, g/cm^3	0,982	1,014	1,05	0,9165
Refractive index at 25°C, kg/l	1,4840	1,4468	1,4112	1,4742
Water content, %	0,0014	0,0029	0,0012	0,00

Table 2. Plasticizers characteristics

Characteristics	*Values*
K-WERT value, SR EN ISO 1628- 1, 2/2003	70
Oxidation-reducing substances, ml $Na_2S_2O_3$ 0,01M, EUROPEAN PHARMACOPOEIA, ED. 6	0,41
Moisture and volatiles, % ASTM D 3030/2000	0,052
Residue on the sieve of 0,063 mm, % SR EN ISO 4610/2003	97,1

2.2 Sample preparation:

Samples were prepared via usual PVC compounding technology. The raw materials were dry blended into Fluid-Mischer apparatus with capacity of 5 l, and then melt blended and granulated on extruder with two screws, type Ko-Buss. The work temperature was 105°C/135°C/145°C.

It were performed three recipes of medical grade PVC such as:

- Recipe P1 with plasticizer Proviplast 01422 [bis(2-(2-butoxyethoxy)ethyl)adipate],
- Recipe P2 with plasticizer Proviplast 2624 (tributyl 2-acetylcitrate),
- Recipe P3 with plasticizer DEHP [Di-(2-EthylHexyl) Phthalate],
- Recipe P1AM is recipe P1 compounded with silver salts nanoparticles 10%wt,
- Recipe P2AM is recipe P2 compounded with silver salts nanoparticles 10%wt,
- Recipe P3AM is recipe P3 compounded with silver salts nanoparticles 10%wt.

The granules obtained were melting pressed into a Brabender laboratory press at temperature 160°C and pressure 250 barr, resulting plates of 1mm thickness. Specimens were taken from plates in order to be tested.

3. Characterisation of samples

Physical-mechanical properties were determined as following:

- Melt flowing (MFI) of samples were determined on Thermo Haake – Meltflixer 2000 apparatus.
- Thermal properties were analyzed by DSC, on the apparatus METLER – TOLEDO. Thermal properties were analyzed by DSC measurements of temperature and heat flow associated with transitions in the control samples formulations, depending on temperature and time in a controlled atmosphere, within Mettler-Toledo 823 apparatus. The purpose of this test was to compare the thermal properties in order to select the most appropriate plasticizer to achieve effective and optimum recipe for application in antimicrobial medical devices. It were assessed the fluctuations of glass transition temperature of PVC recipes plasticized with addipates, citrates and DEHP plasticizers. In order to perform analyzes, samples every of 10 mg were taken, and were placed in capsule of aluminum, which in turn were placed in DSC apparatus. Work temperatures ranged between 30 °C - 250 °C and the heating rate was 10 °C / min. Samples were heated and cooled in capsules unit, under controlled conditions. DSC analysis provides data about the temperature of the PVC processing receipts [8].
- FTIR analyses were performed on spectrophotometer FTLA 2000-104 (ABB Canada) with accessory Smart DuraSampl IR HATR – (Horizontal Attenuated Total Reflectance), accessory with a laminated–diamond crystal. The spectra were the average of 22 scans recorded at a resolution of 4 cm^{-1} in the range from 4000 to 600 cm^{-1}.
- Tensile strength – determinations were performed on dinamometer FT 10.

- Elongation at break - determinations were performed on dinamometer FT 10,
- Hardness Shore A – determinations were performed on durometer Shore,
- Water absorbtion was tested according to SR EN ISO 62:2008, on circular test specimens with a diameter of 20 mm and thickness of 1 mm. Test specimens were weighed initially on analytical balance with an accuracy of ± 0.1 mg and they were introduced in Memmert oven with air circulation at 50°C for 5 days. After removing from the oven, samples were dried and weighed. Specimen mass variation was expressed as a percentage [10]. The purpose of this determination is to evaluate the behavior of tubular medical devices in contact with biological fluids. For each specimen, the percentage change in mass *c* was calculate relative to the initial mass by using the appropriate formula:

$$c = \frac{mf - mi}{mi} \; x \; 100\% \qquad (1)$$

mi is the mass of the specimen, in mg, after initial drying and before immersion,
mf is the mass of the specimen, in mg, after immersion.
- Plasticizer migration,
- Contact angle,
- Evaluation of *in vitro* cytotoxicity of the polymer recipes performed on cell culture Vero using cultivation techniques in suspension within DME medium (Dulbecco's Medium Essential), which contained 10% fetal bovine serum.

4. Results and discussion

Analysis of melt flow properties of experimental recipes aimed the assessing materials behavior under the influence of temperature. Studying of the properties of the melt flow polymer correlated with their density, is very important for assessing energy consumption and to optimize operating parameters for industrial processing. It was determined melt flow index (MFI) for the recipes P1, P2, P3 P1AM, P2AM, P3AM. The results are shown in Figure 1.

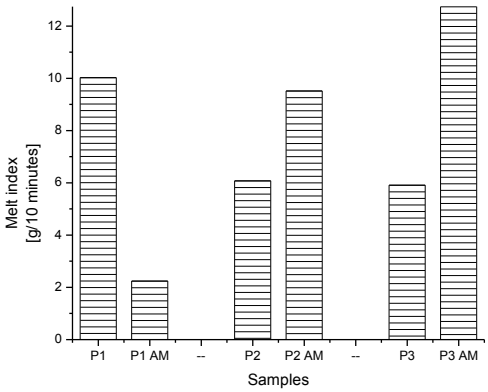

Fig. 1. Variation of melt index in medical grade plasticized recipes of PVC

Except to P1AM, it can observe that presence of nanoparticles of silver salts in the recipes lead to increase of MFI, in comparison with initial samples.

Figure 2 present the DSC termograms for the PVC samples, plasticized in concentration of 30% with low molecular plasticizers: addipates (sample P1), citrates (sample P2) and di-2-ethyl-hexyl phthalate (sample P3).

Recipes P1, P2 and P3 those low molecular plasticizer content was 30% wt, were analyzed by DSC. Samples were heated with rate of 10 ^{o}C/min until temperature raised to 240^{o}C. Transition observed in the range of 51.79^{o}C – 75.88^{o}C represent glass transition (T_g) of recipes (Table 3). Range of glass transition temperatures varies with plasticizer and antimicrobial agent loading. It can observe that molecular mass and melt viscosity of citrates (higher than those of addipates and DEHP), influence directly transition temperature by rising to 51,79^{o}C. Also, addipates present similar influence to transition temperature, to DEHP. But overall behavior transition temperature of the samples studied, is almost like.

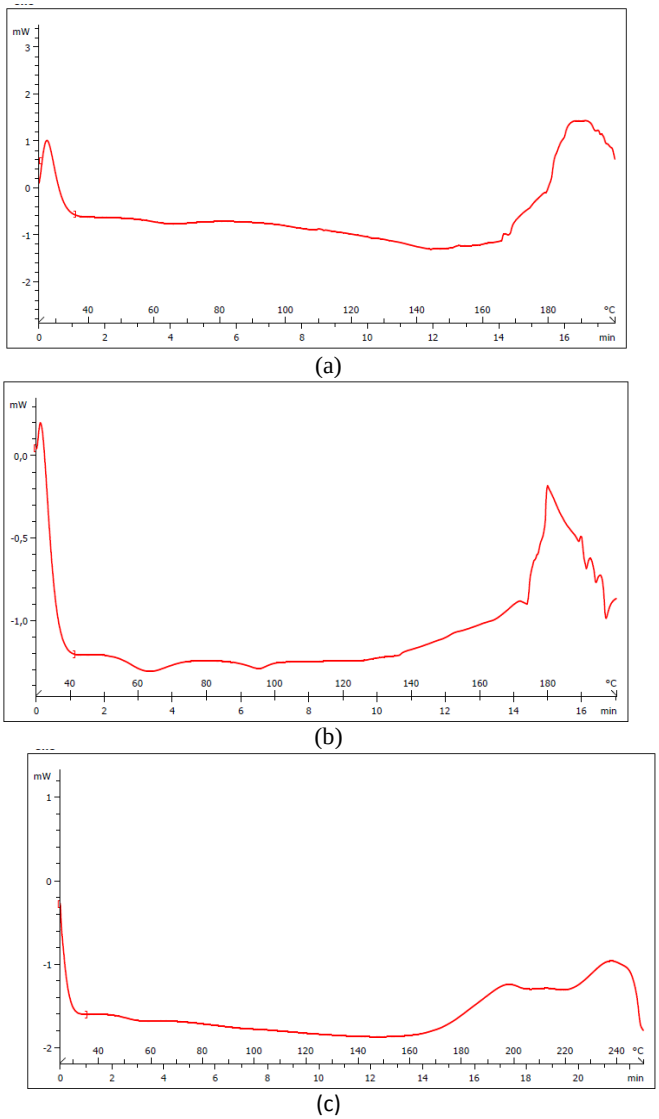

Fig. 2. DSC analysis of plasticized PVC samples: a) sample P1, b) sample P2, c) sample P3.

It can be observed a sligth change of baseline that accompanies the glass transition of the samples. The values of glass transition temperature are low because of a relative high content of plasticizers: 30% primary plasticizer (addipates, citrates and DEHP) and 1.6% soy bean oil, as secondary plasticizer. That means the plasticization of PVC using the three plasticizers above mentioned in high concentration can lead to widened relaxation temperature width in glass transition. Recipes P2 and P3 exhibit Tg lower, that explain an incresed plasticizing effect in comparison with P1 those addipates plasticizers allow only a reduced movement of molecular chains under heating.

Heat specific temperature (ΔCp) low values is due to plasticizer and antimicrobial content, that determine moderates processing work temperatures.

FTIR analysis confirm the qualitative similarities between chemical structures of the recipes, due to components. Characteristic bands of PVC and additives are presented in figure 3. It can observe that bands in the range 710–589 cm^{-1} corresponding to C-Cl, and bands form range of 1464–1425 cm^{-1} are attributed to C-H bonds in PVC. Instead, bands from 1480–1450 cm^{-1} and 1800–1690 cm^{-1} are attributed to methyl (–CH3) and carbonyl groupes of the plasticizers.

Table 3. Glass transition temperatures of plasticized PVC recipes obtained by DSC

Recipe	*Tg [°C] (initial temp.)*	*Tg [°C] (glass transition temp.)*	*Tg [°C] (final temp. of glass transition Tg)*	*ΔCp [J/g°C] (specific heat capacity gradient before and after T_g)*
P1	67.02	70.11	74.28	0.302
P2	51.01	55.06	61.12	0.276
P3	47.81	51.79	59.85	0.183
P1AM	71.05	75.88	80.04	0.389
P2AM	68.12	70.02	67.13	0.301
P3AM	63.31	67.23	61.24	0.279

The range of spectra (figure 3) is composed as a mean of 22 scans with resolution of 4 cm^{-1} in the wavelength range of 4000 – 600 cm^{-1}.

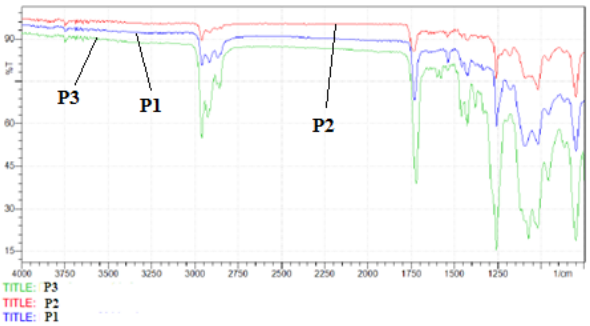

Fig. 3. FTIR spectra of P1, P2, P3 in the range of (4000 – 600) cm^{-1}

Analysis of the physical-mechanical properties of the recipes is presented in the next figures.

Hardness Shore exhibit values required from European Pharmacopoeia [9]. The plasticizer forms links with polymer molecules and acts as spacer between molecules of the polymer. Due to this linkage plasticizer has great effects on the mechanical properties of the polymer.

All recipes are similar, but they differ by the type of plasticizer. Mechanical properties reflect the influence of the plasticizer, as hardness Shore increase in P1AM and P3AM compared to initial recipes. Mechanical properties of the three recipes exhibit values accepted for materials with medical applications, which are subjected to repeated bending and contact with body fluids.

Tensile strength exhibit similar values in the recipes P1, P2, P3, but adding antimicrobial agent determined decrease of values in recipes plasticized with citrate and DEHP (figure 4).

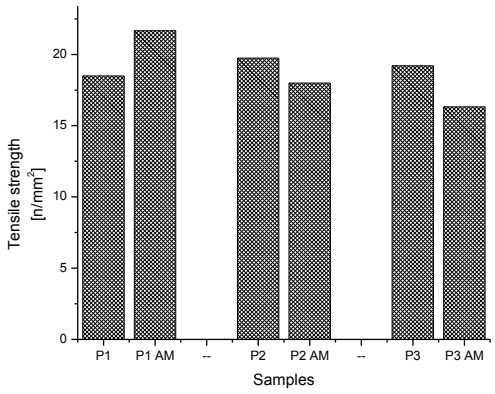

Fig. 4. Variation of tensile strength in medical grade plasticized recipes of PVC

Elongation at break exhibit higher values in recipe P3 and P3AM due to citrate plasticizer, instead decrease in recipe plasticized with DEHP and addipate. Filling recipes with antimicrobial agent lead to decrease of tensile strength in recipes plasticized with addipates and DEHP (Fig. 5).

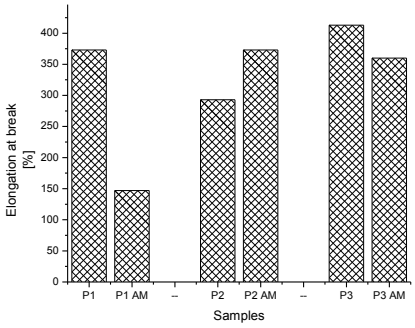

Fig. 5. Variation of elongation at break in medical grade plasticized recipes of PVC

All the recipes contain the same materials in the same quantities. They are different by the type of plasticizer. It can see that the density of plasticizers are different and influences the mechanical properties. Recipe P1 with addipates plasticizer exhibit the greatest hardness Shore and tensile strength, respectively the smallest elongation at break. Instead the recipe plasticized with DEHP exhibit the greatest elongation at break (Fig. 6).

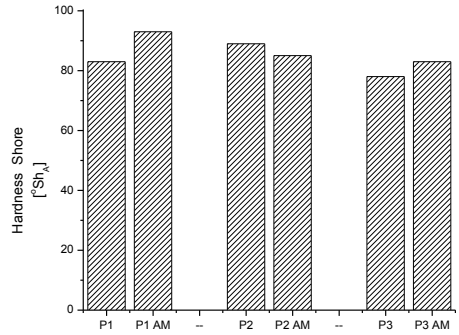

Fig. 6. Variation of hardness Shore in medical grade plasticized recipes of PVC

Nevertheless, the values obtained for mechanical properties are similar between recipes. It seems that recipe P2 plasticized with citrates is more likely to P3 plasticized with DEHP. But the results are very indicated to use the three materials in medical application such as medical devices catheters or drains.

Measurements to determine the contact angle between the surface and water plasticized PVC plate were performed at room temperature droplet method ("Sessile drop method") at 20°C within 30 seconds after placing liquid drops of 5 μ L plate surface. This time is long enough that the contact angle of the droplet to reach equilibrium value and short enough so as evaporation losses are negligible. It was used the equipment CAM 101, provided by digital camera C200-HS type KSV Finland to record the drop image. The value of contact angle for surface plasticized PVC recipe, is shown in Table 4.

Table 4. Contact angle of recipes

Recipe	*Contact angle [°]*
P1	93
P1AM	101
P2	96
P2AM	98
P3	89.7
P3AM	111

It is noted that the value of contact angle value is relatively low, which indicates that the recipe of PVC plasticized is of hydrophobic nature.

Water absorbtion results were presented in the next table.

Table 5. Water absorbtion of plasticized PVC recipes

Recipe	*Water absorbtion %*
P1	0.054
P1AM	0.029
P2	0.003
P2AM	0,033
P3	0,003
P3AM	0,001

It was determined plasticizer loss by immersing circular samples of 30 mm diameter and 1 mm thickness, in saline solution five days. Mass loss of the specimen plasticized PVC plasticizer migration is a measure of plasticizer migration. The results are shown in Table 6.

Table 6. Plasticizer loss

Recipe	*Initial mass [g]*	*Final mass [g]*	*Plasticizer loss [g]*
P1	24,25	24,22	0,03
P1AM	34,78	34,75	0,02
P2	38,43	38,41	0,02
P2AM	35,32	35,31	0,01
P3	34,76	34,73	0,03
P3AM	37,88	37,86	0,02

Also, films from recipes P1, P2, P3 were tested by UV-VIS spectrometry in order to highlight the loss of plasticizer after immersion in saline solution of PVC samples. In the next figures are presented UV-VIS curves registered on recipes initial and after immersion (Fig. 7).

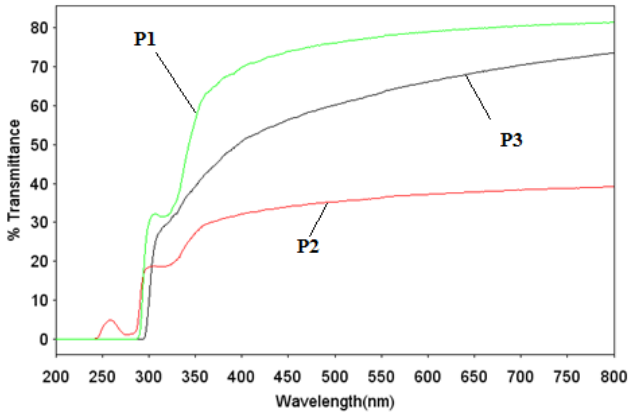

Fig. 7. UV-VIS transmittance through medical grade plasticized PVC

According to UV-VIS spectra, after immersion in saline solution, recipe P1 plasticized with addipate loses 5% of transmittance, recipe P2 behave similar as initial so was no loss of citrate, and recipe P3 loses 17,1% of transmittance. The results show that DEHP migrate in the

higher quantity in saline solution during immersion (fig. 8).

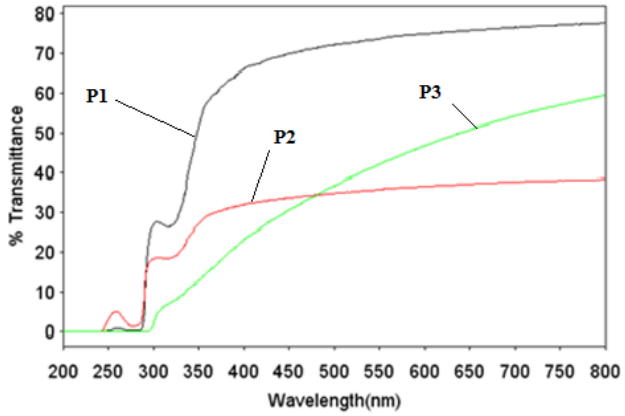

Fig. 8. UV-VIS transmittance through medical grade plasticized PVC after immersion in saline solution.

The results of MTT assay are presented in next figures.

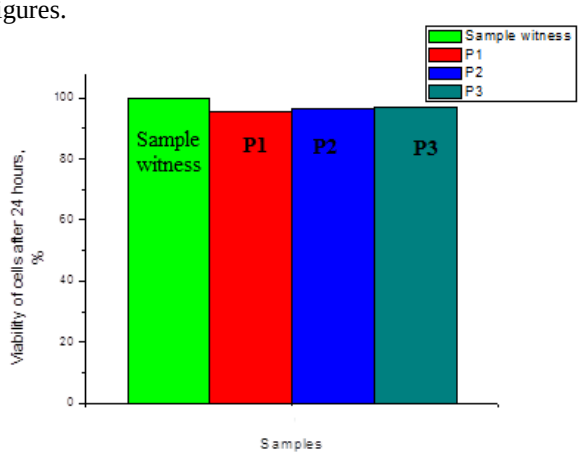

Fig.9. Viability of cells from culture medium after 24 hours in contact with samples

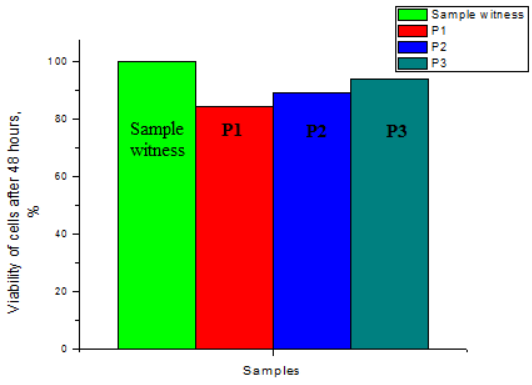

Fig. 10. Viability of cells from culture medium after 48 hours in contact with samples

As a prerequisite for the use of polymers in medical applications is the property of biocompatibility it were presented methods for *in vitro* cytotoxicity testing. To test the *in vitro* biocompatibility of the polymer recipes they were compared to the control sample, using the cell culture method, a technique of growing in suspension. There was no change in appearance compared to control cells. In addition, it was observed that cells adhered to the surface of samples, demonstrating biocompatibility of the polymeric recipes. Samples analyzed showed no cytotoxicity. The stability of the samples (in terms of compositional) in culture medium was high in all samples.

5. Conclusions

In the present work it was investigated the influence of the type of plasticizers and the presence of antimicrobial additives to achieve medical grade PVC recipes. It were performed compounding of PVC with several additives: stabilizers and plasticizers, following usual protocol as, dry blending, melt blending at 170^{o}C, granulating and pressing at 160^{o}C to obtain plates for sampling in order to analyze physical-mechanical properties.

Experiments carried out have had the purpose of selecting biocompatible plasticizer, in order to perform medical grades recipes of PVC for use in medical devices with antimicrobial properties, type urinary catheter.

DSC measurements were used to highlight the effect of type of plasticizers on glass transition of PVC recipes.

FTIR analyses demonstrates similarities between the chemical structure of recipes due to the raw components.

Antimicrobial properties has an important role in plastic medical devices. Plasticizers in recipes offer better quality of mechanical properties such hardness, tensile strength and elongation at break, required to medical devices applications. The values obtained offer important information especially about biocompatibility and flexibility so necessary in medical device using.

The plasticizer forms links with polymer molecules and acts as spacer between molecules of the polymer. Due to this linkage plasticizer has great effects on the mechanical properties of the polymer.

As it were observed, silver salts nanoparticles acts as fillers in PVC compounding. Their influence on processing conditions and melting behavior of the recipes could be observed on values obtained in physical-mechanical properties.

Mechanical properties reflected the difference between recipes due to PVC plasticizers. The values obtained have shown that the use of addipates and citrates plasticizers instead of DOP, does not significantly influence the mechanical properties. Recipes plasticized with addipate shows tensile strength and elongation at break similar to recipe plasticized with citrate, but greater than recipe plasticized with DOP. Hardness Shore of recipe plasticized with citrate was greater than of recipes plasticized with addipate and DOP. Instead antimicrobial recipes lead to improvement of tensile strength of recipe plasticized with addipate and increase elongation at break of recipe plasticized with citrate. Hardness Shore increased in recipes plasticized with addipate and DOP when recipes were loaded with antimicrobial agent.

Loss of plasticizer was significantly in recipe plasticized with DOP and reduced in recipe plasticized with addipate. Recipe P2 lose not plasticizer citrate during immersion in saline solution.

Contact angle determined revealed that recipes P1, P2, P3 exhibit hydrophilic properties of the surfaces. Also, antimicrobial recipes presented contact angle similar to hydrophilic materials.

The results obtained fall within the limits of the European Pharmacopoeia, so as experimental antimicrobial recipes can be used in the manufacture of tubular prosthetic medical devices.

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