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Surface characterisation of bags for total parenteral nutrition by tensiometry and atomic force microscopy

Nicola Realdon [a,*], Lucio Zennaro [b], Francesco Perin [a], Antonio Bettero [a], Silvia Bortoluzzi [b], Adelio Rigo [b], Enrico Ragazzi [a]

[a] *Department of Pharmaceutical Sciences, University of Padova, via F. Marzolo 5, Padova I-35131, Italy*
[b] *Department of Biological Chemistry, University of Padova, via C. Colombo 3, Padova I-35121, Italy*

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Abstract

Bags made of poly-ethylene and poly-vinylchloride and of the copolymer ethylene–vinylacetate were used as containers of perfusion solutions for total parenteral nutrition. The bags were characterised by tensiometry (free energy and its polar and dispersed components) and atomic force microscopy (AFM) before and after various periods of storage of solutions for total parenteral nutrition containing L-aminoacids, electrolytes or glucose. In most of the cases, after storage of these solutions, tensiometric characterisation and atomic force microscopy analysis of the internal surface of bags showed deep modifications which highlight the adsorption of the solutes. The changes of surface characteristics were found to depend on the time of contact, the wettability of the polymer and the compounds present into the solutions, while their concentration has a negligible effect. Generally, the aminoacid solutions produced a higher increase in the polar component even after short storage times. Poly-ethylene and the copolymer ethylene–vinylacetate showed a greater inertia if compared with the poly-vinylchloride bags.

Keywords: Total parenteral nutrition; Tensiometry; Atomic force microscopy; Poly-ethylenevinylacetate; Poly-ethylene; Poly-vinylchloride

1. Introduction

Injectable solutions for Total Parenteral Nutrition (TPN), containing L-aminoacids, electrolytes and glucose, are commonly sold as medicinal specialities in glass containers. Many studies have been carried out to evaluate the possibility of commercialising these solutions and/or their mixtures, packaged directly in plastic bags. As a result of these studies bags made of plastic materials such as copolymer ethylene–vinylacetate (EVA), poly-ethylene (PE) and poly-vinylchloride (PVC) (Pignataro, 1996; Morra and Cassinelli, 1997) are being used more and more often in the manufacture of containers of perfusion solutions. A problem, associated with the use of these plastic bags, is the loss of solution components through adsorption on the inner surface of the container. This phenomenon has been reported to occur for several drugs, such as diazepam, insulin and organic nitrates for which significant losses have been noted after storage of perfusion liquid in TPN bags (Weisenfeld et al., 1968; Parker et al., 1979; Yuen et al., 1979; Roberts et al., 1980; Kowaluk et al., 1984), the adsorption of solution components being dependent on contact time and wettability of the polymers.

* Corresponding author. Tel.: +39-049-8275339; fax: +39-049-8275366.
E-mail address: nicola.realdon@unipd.it (N. Realdon).

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From these premises and the technological needs, by the use of tensiometry and atomic force microscopy (AFM) (Hansma and Tersoff, 1987; Mc Pherson et al., 2000) an experimental protocol was planned to evaluate the effects of storage of TPN solutions on the characteristics of the inner wall of bags made by plastic materials widely used in the medical field.

2. Materials and methods

2.1. Materials

Water for injectable preparations, isopuramin 10% (L-alanine 7.00 g/l, L-arginine 11.7 g/l, L-phenylalanine 7.90 g/l, glycine 6.40 g/l, L-isoleucine 8.00 g/l, L-histidine 5.10 g/l, L-leucine 10.60 g/l, L-lysine 11.50 g/l, L-methionine 7.20 g/l, L-tyrosine 0.30 g/l, L-threonine 9.50 g/l, L-tryptophan 3.20 g/l, L-valine 11.60 g/l), and the solutions of calcium gluconate, magnesium chloride, potassium phosphate, potassium lactate, sodium chloride were purchased by Bieffe Medital, Milan, Italy. Solutions of glucose (70%) were obtained by S.A.L.F., Bergamo, Italy.

The EVA bags were supplied by Sifra (Verona, Italy), the PE bags by Bieffe Medital (Milano, Italy) and the PVC bags by B. Braun (Mirandola, Italy).

2.2. Methods

Tensiometric measurements were carried out by a tensiometer G40 (Kruss GmbH, Hamburg), equipped with G1041 Microcamera, a manual dosimeter for surface and interface tension measurements, a manual dosimeter for contact angle, a G1023 automatic dosimeter for dynamic contact angle, a TD-211 temperature monitor and with an Hamilton 1750 TLL syringe. The "G402.06" and "Pendant Drop Analysis" softwares were used.

A Mettler balance AE 100, equipped with a ME-33360 kit and ME-210260 body immersion was used to carry out density measurements of the solutions.

AFM measurements were carried out at room temperature using an atomic force microscope AUTOPROBE CP (Park Scientific Instruments, Sunnyvale, CA), equipped with a contact head, an intermittent contact head, and a 5-μm scanner.

2.2.1. Surface characterisation by tensiometric measurements

Four samples (75 mm × 25 mm) were taken from different areas of each bag and washed thoroughly with deionised water and finally with water for injectable preparations and dried. The outer surface was made to adhere to a microscopy glass slide. Water and diiodomethane were used as standard liquids (Fowkes, 1964; Neumann and Good, 1979). A drop of a standard liquid (diameter 2–6 mm) was applied on the surface of the sample and the contact angle was measured (Fowkes, 1964; Owens and Wendt, 1969; Wu, 1971; Neumann and Good, 1979; Gaydos and Neumann, 1987). The acquired angle values were elaborated using the Wu method (Owens and Wendt, 1969; Wu, 1971) to obtain the polar (PC) and dispersed (DC) components the sum of which gives the surface free energy (SFE).

The tensiometric characteristics of the material can be represented in terms of TVS® index (SFE, PC and DC), see Fig. 1, according to the parametric model developed at the "C. A. Benassi" Chemical Cosmetology Centre of the University of Padova. This model represents integrated "tensiometricprints" and SFE contextually with its related DC and PC components of a material or biological substrate and their tensiometric affinity (Bettero et al., 1998a,b).

The SFE, PC and DC values reported in this paper are the mean values obtained from the measurements carried out on four samples of bags.

2.2.2. Surface characterisation by atomic force microscopy

All the samples were investigated in air using contact or tapping mode techniques. Silicon Ultralevers type C-D (Park Scientific), with resonance frequency of 300–400 kHz, were used for tapping mode. Silicon nitride (Si_3N_4) microlevers, type A, with a nominal spring constant of 0.05 N/m, were used for contact mode. Scan rates were in the range of 2–4 Hz. Four samples (10 mm × 10 mm) were cut from each bag. Before cutting, the bags were washed thoroughly with deionised water and with water for injectable preparations. The samples were dried carefully with a nitrogen stream and stored in closed and sterile containers until they were mounted on the AFM sample holder. A set of nine images of the inner surface was acquired for each sample analysed, usually at three different posi-

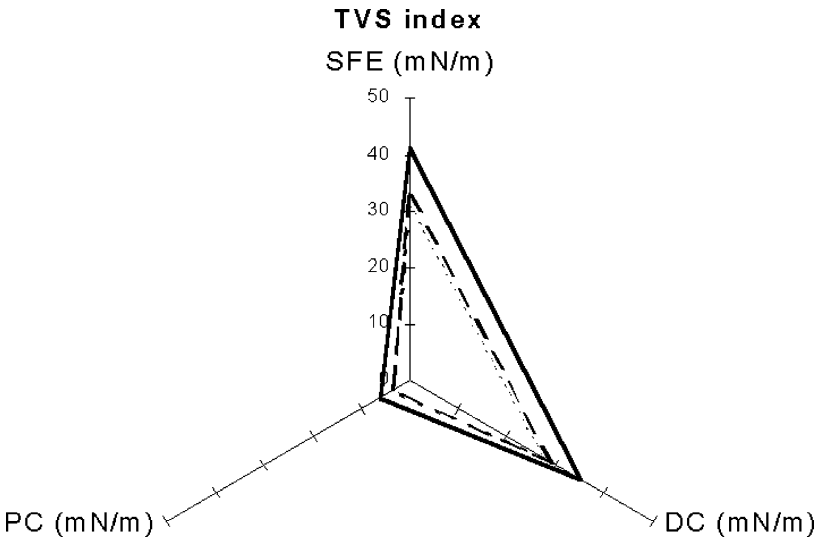

Fig. 1. Values of the TVS® index of EVA (—), PE (– – –) and PVC (- - -) plastic bags.

tions, namely at the centre, at the upper left corner and at the lower right corner of the sample. The surface mean height and the surface mean roughness (R_{rms}) were used as parameters to characterise the surfaces of the samples ($R_{rms} = \sqrt{\sum_{i=1,N} (z_i - \langle z \rangle)^2 / N - 1}$, where N is the number of Pixels in the image, z_i are the heights of each pixel, and $\langle z \rangle$ is the mean height calculated over 512 points per scan line and 512 scan lines per image).

All the images, and the mean heights and roughness values reported refer to that ones obtained using contact mode technique and Si_3N_4 probes (microlevers, type A).

2.2.3. Tensiometric characterisation of the perfusion solutions

The measurement of surface tension of the perfusion solutions was carried out by the "pendant drop" method. A needle of 1.5 mm of diameter was used. A mean value of the surface tension was obtained from 15 extractions of the drop profile by using the "Pendant Drop Analysis" calculation programme (Girault et al., 1984; Anastasiadis et al., 1987; Hansen and Rodsrud, 1991). The PC and DC values of the perfusion solutions were calculated according to many authors (Wu, 1971; Girault et al., 1984; Anastasiadis et al., 1987; Hansen and Rodsrud, 1991) by measuring the contact angle at equilibrium on PE film, as standard solid, washed thoroughly with deionised water and finally with water for injectable preparations and dried.

2.2.4. Formulation and filling of bags

The composition of the three types of TPN solutions (electrolytes, glucose and aminoacids) used for testing of EVA, PE and PVC bags is reported in Table 1. Water for injectable preparations was used in the controls. The filling of the bags was carried out by dropping

Table 1
Composition of TPN solutions

Electrolyte solutions				
Composition (mEq/l)	E 1	E 2	E 3	
Calcium gluconate	139.2	278.4	696	
Natrium chloride	100	200	500	
Magnesium sulphate	166.1	332.2	830.5	
Potassium lactate	12	24	60	
Potassium phosphate	40	80	200	
Glucose solutions				
Composition (g/l)	G 1	G 2	G 3	
Glucose	100.1	301	500.5	
Aminoacid solutions				
Composition (ml/l)	A 1	A 2	A 3	A 4
Isopuramin 10%	250	500	750	1000

the perfusion solutions under a class A laminar flow hood. The filled bags were stored at $5 \pm 1\,^{\circ}C$, under controlled conditions for various periods of time.

3. Results

3.1. Superficial characterisation of TPN bags

The TVS® indexes characterising the internal surface of EVA, PE and PVC bags were calculated from the averaged values of SFE, DC and PC components and are reported in Fig. 1. From this figure, it appears that EVA bags show the higher TVS values, while PE and PVC are characterised by lower and similar TVS indexes.

In Fig. 2, the AFM images of the bag surfaces before filling with TPN solutions are reported. From this figure, it appears that the AFM images of EVA and PE bags are characterised by a close surface roughness ($z_{rms} = 25.5 \pm 5.6$ Å and 30.2 ± 1.9 Å, respectively), and by similar surface mean height (25.7 ± 1.1 Å and 35.1 ± 0.3 Å, respectively). In the case of PVC, the mean height and roughness values were noticeably higher ($z_{rms} = 124.3 \pm 13.1$ Å, mean height $= 192.1 \pm 24.6$ Å).

3.2. Effect of TPN composition on the surface characteristics of bags

Various trials of bags were filled with three types of TPN solutions (electrolytes, glucose and aminoacids). Each type of TPN solution was tested at three different concentrations, see Table 1. The bags, together with a trial of bags filled with water for injectable preparations as a control, were stored at $5 \pm 1\,^{\circ}C$

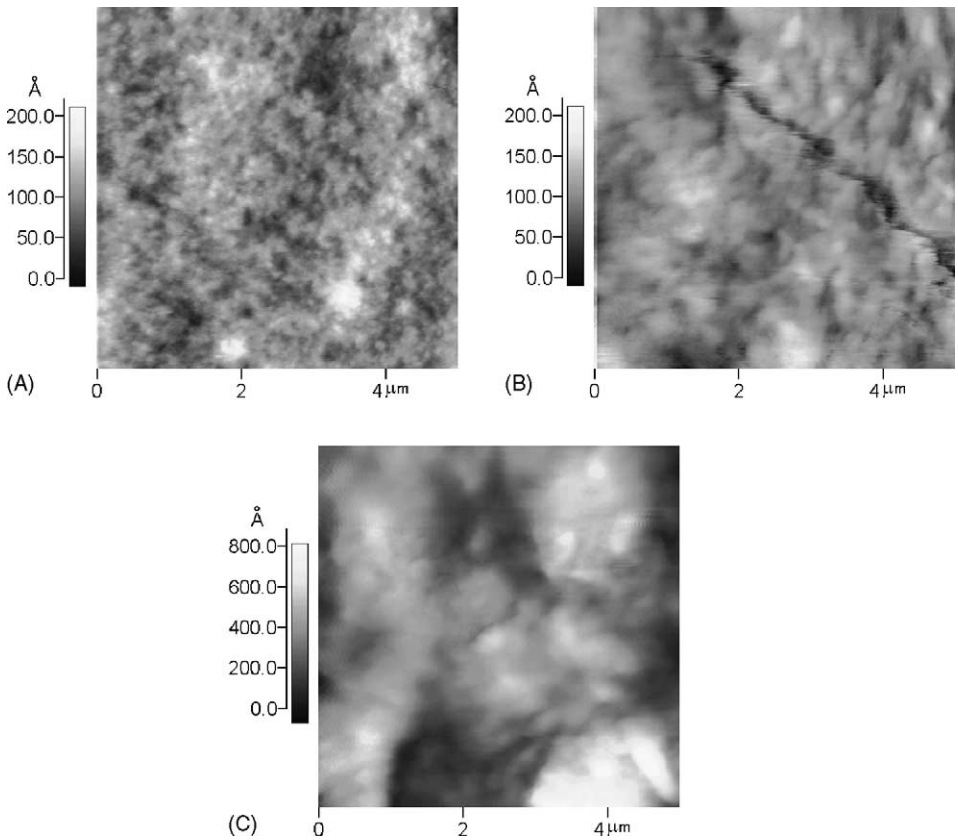

Fig. 2. AFM images of the inner surface of plastic bags: (A) EVA, (B) PE, (C) PVC before filling with the TPN solutions.

for periods of time ranging from one week to six months. No significant change of the surface characteristics (tensiometric and AFM) as a consequence of the storage of injectable water were found in the control bags.

The storage of TPN solutions usually brought to a modification of the surface characteristics, in particular of PC components, while no appreciable change of the DC component was observed. According to these results only the PC component of SFE was further considered.

3.2.1. Bags in EVA

The EVA bags are characterised by a PC value of about 6 mN/m and for the various TPN solutions this value increased of about 20% after one month of storage. For glucose and electrolyte solutions no further change of the PC value with time was observed, while in the case of aminoacid solutions a progressive increase of these values up to 50–60% of the initial value was measured after six months of storage, see Fig. 3. In all cases no evident change of the PC component on the concentration of TPN solutions was observed.

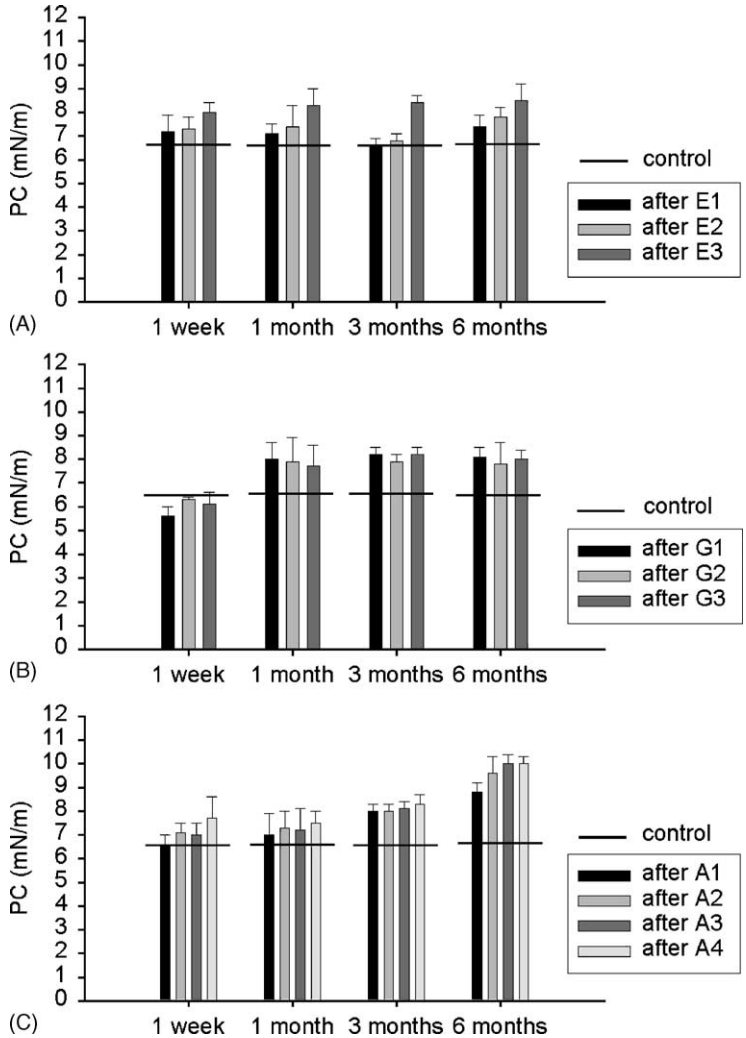

Fig. 3. PC (mN/m) values of the inner surface of bags in EVA after filling and storage with solutions of increasing concentrations of: (A) electrolytes, (B) glucose, (C) aminoacids.

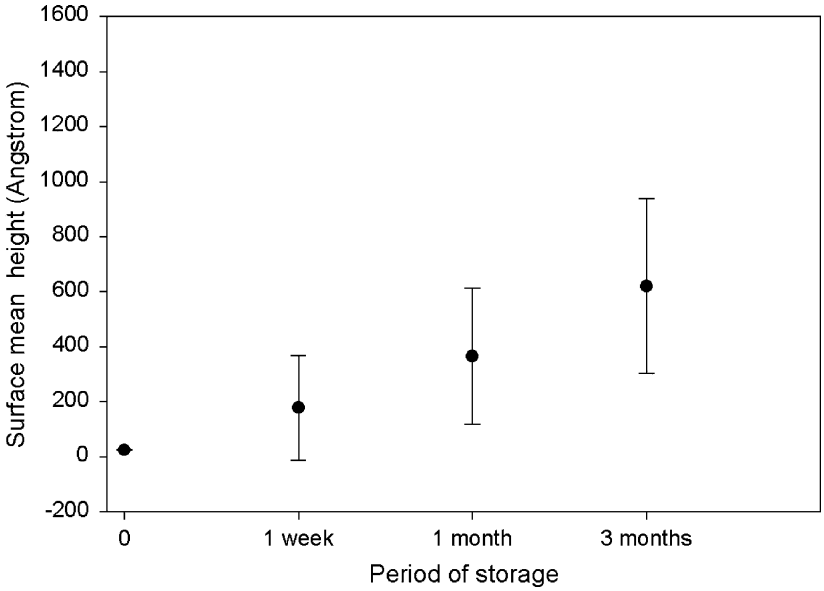

Fig. 4. Surface mean height of the inner surface of EVA bags after storage of the aminoacid solution (concentration A4).

The inner surface of the bags, after storage of TPN solutions, at AFM analysis generally appeared homogeneous with a relevant and progressive increase of the surface mean height with time. In particular, in the case of aminoacid solutions the average heights increased more than one order of magnitude after three months of storage, see Fig. 4.

3.2.2. Bags in PE

The tensiometric analysis revealed that the PC component value of this material is about 3 mN/m before storage of TPN solutions and increased of about 20% after one month of storage of these solutions, independently of their composition. No further changes were observed for the glucose solutions while for the electrolyte and aminoacid TPN solutions the PC values increased up to 60–80% after six-month storage, see Fig. 5.

The AFM analysis of the bags after storage of TPN solutions show strong modifications of the surface. In particular, the surfaces appeared not homogeneous due to the presence of large agglomerates. In the case of storage of glucose solutions, the smallest increase of the surface mean heights was measured. No dependence of the surface mean height on storage time and on the composition of the TPN solutions was observed for PE bags.

3.2.3. Bags in PVC

The storage of TPN solutions in PVC bags affects strongly the PC component of the TVS® index. In particular, in the case of glucose and aminoacid solutions the PC values, starting from an initial value of 3.6 mN/m, increase more than 80% just after a week of storage, reaching 120–160% after six months of storage. Even in the case of electrolyte solutions, a similar strong increase was observed, but only after six months of storage, see Fig. 6. In any case, the increase was found independent of the concentration of the TPN solutions.

The surface of the PVC bags after storage of glucose and electrolyte solutions appeared at the AFM analysis relatively homogeneous notwithstanding the strong increase of the surface mean height. In the case of storage of aminoacid solutions, the increase of the height was paralleled by the formation of large aggregates characterised by a height of about 2000 Å, see Fig. 7.

4. Discussion

Tensiometric analysis of the inner surface of plastic bags allows the evaluation of the modification of the bag materials due to the storage of TPN solutions

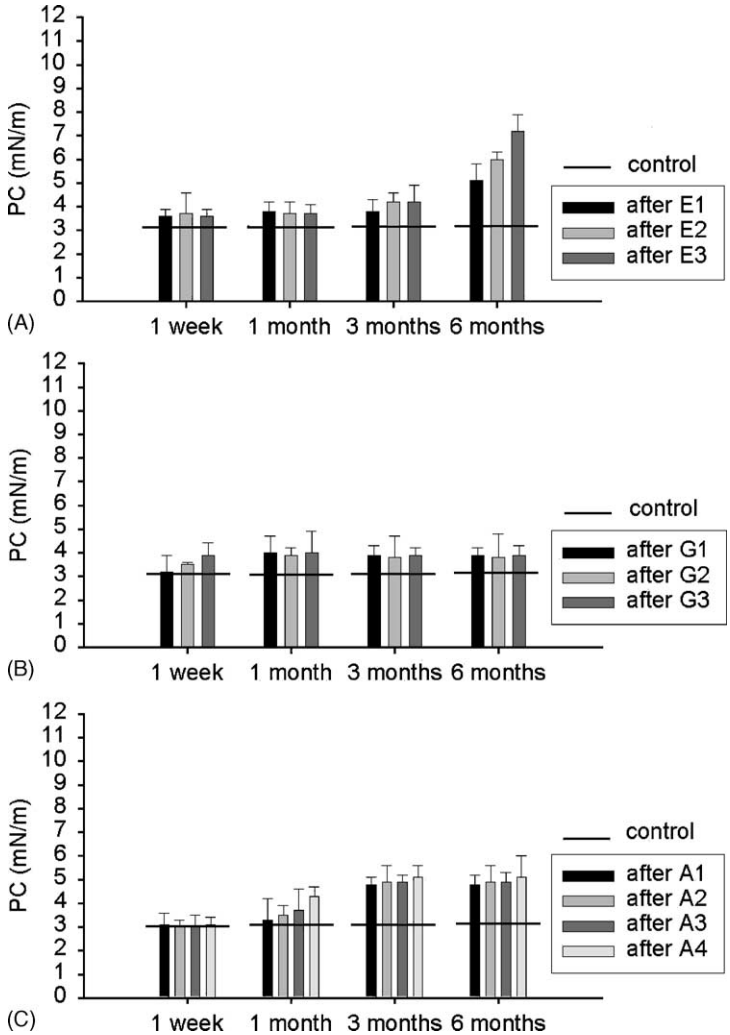

Fig. 5. PC (mN/m) values of the inner surface of a bag in PE after filling and storage with solutions of increasing concentrations of: (A) electrolytes, (B) glucose, (C) aminoacids.

by means of the DC and PC components of TVS indexes® (Bettero et al., 1998a,b). Notwithstanding the structural low homogeneity of the surface of plastic material surface due to the manufacture, a relatively small change of TVS values have been observed both between bags of identical material and, to a lesser extent, between different areas of the same bag (standard deviations 5–10%). For the materials used in this study, the tensiometric profile is a characteristic of the polymer used and the dispersed component predominates the polar one. This is in accordance with the poor wettability of the surface of the polymers we took into consideration (see Fig. 1 and Table 2).

The storage of TPN solutions in the bags usually lead to a change of the PC values while the DC values practically do not change, indicating a modification of the inner surface of the bags due to the components of TPN solutions. In fact on contact with strongly polar solutions, such as the TPN aqueous solutions, a prevalence of cohesion forces among the molecules of solution over their adhesion forces to the polymer substrate has been found. This is evident from a comparison be-

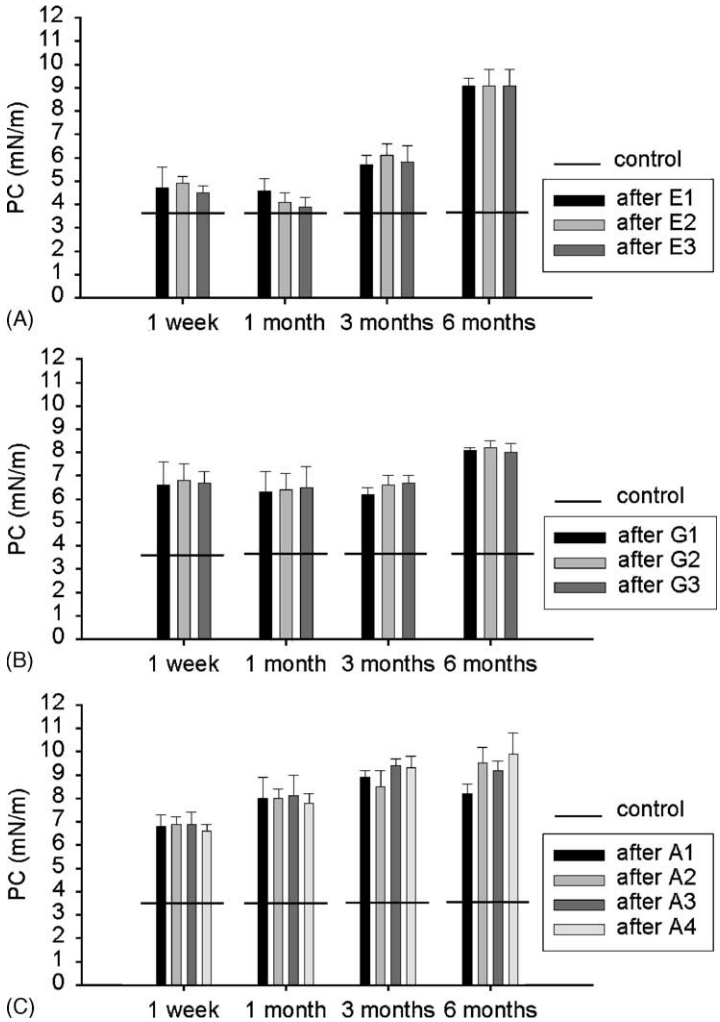

Fig. 6. PC (mN/m) values of the inner surface of a bag in PVC after filling and storage with solutions of increasing concentrations of: (A) electrolytes, (B) glucose, (C) aminoacids.

Table 2
TVS® indexes of the bags and the TPN solutions

	SFE (mN/m) (±S.D.)	DC (mN/m) (±S.D.)	PC (mN/m) (±S.D.)
Bags			
EVA	41.1 (0.7)	35.1 (1.7)	6.0 (1.4)
PE	32.4 (0.4)	29.3 (0.3)	3.1 (0.4)
PVC	30.6 (0.6)	27.6 (0.8)	3.0 (0.7)
TPN solutions			
A 4	68.2 (0.3)	30.1 (0.3)	38.1 (0.5)
E 3	74.2 (0.6)	30.8 (0.5)	43.4 (0.4)
G 3	74.2 (0.9)	32.6 (0.9)	41.6 (0.5)

tween TVS indexes of the bags and those of the TPN solutions, see Table 2. Despite this, following storage over time of various TPN solutions (electrolytes, glucose and aminoacids), modifications of surface characteristics of bags were generally found with an increase in PC value of the surface and therefore of the wettability of the bag's inner surface. The small modifications of the surface of the PE bags observed after storage of TPN solutions may reflect both the higher chemical inertia and the negligible polar characteristics of this polymer with respect to vinyl polymers such as PVC or copolymers containing vinylacetate units (EVA).

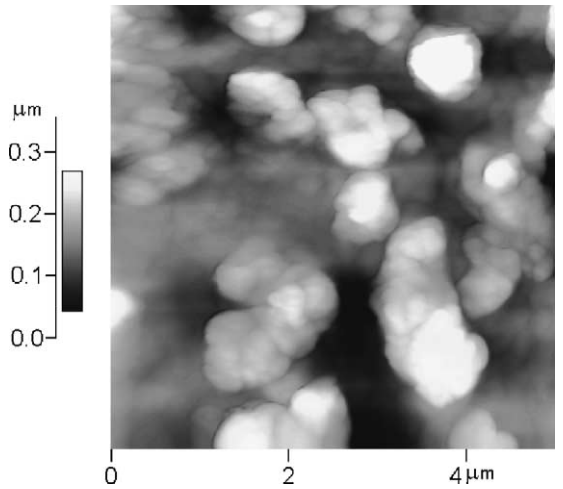

Fig. 7. AFM images of the inner surface of a PVC bag after storage for six months with aminoacid solution (concentration A4).

The modifications of surface due to the adsorption of components of TPN mixtures were found to depend mainly on contact time and on the nature of the components of mixtures rather than on their concentrations.

The AFM images show an increase of the surface mean height after contact with TPN solutions, increase which appears positively correlated with the increase of the PC components of TVS index in most of the cases.

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