# Long Term Stability Evaluation of Prostacyclin Released from Biomedical Device Through Turbiscan Lab Expert

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**Abstract:** Therapeutic guidelines indicate prostacyclin as the first line of treatment in inflammation and vascular diseases. Prostacyclins prevent formation of the platelet plug involved in primary hemostasis by inhibiting platelet activation and, combined with thromboxane, are effective vasodilators in vascular damage.

Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease II guidelines indicates prostacyclins; in particular, Iloprost, as the first therapeutic option for treating peripheral arterial disease. However, therapeutic efficacy of Iloprost has witnessed several drawbacks that have occurred in patients receiving repeated weekly administration of the drug by intravenous infusions. Adverse reactions arose under perfusion with Iloprost for 6 h and patient compliance was drastically decreased. Biomedical devices could provide a suitable alternative to overcome these drawbacks. In particular, elastomeric pumps, filled with Iloprost isotonic solution, could slowly release the drug, thus decreasing its side effects, representing a valid alternative to hospitalization of patients affected by peripheral arterial disease. However, the home therapy treatment of patients requires long-term stability of Iloprost in solution-loaded elastomeric pumps.

The aim of this work was to investigate the long-term stability of Iloprost isotonic solution in biomedical devices using Turbiscan technology. Turbiscan Lab Expert (L'Union, France) predicts the long-term stability of suspensions, emulsions and colloidal formulations by measuring backscattering and transmission of particulates dispersed in solution. The formulations were evaluated by measuring the variation of physical-chemical properties of colloids and suspensions as a function of backscattering and transmission modifications. In addition, the release profile of Iloprost isotonic solution from the biomedical device was evaluated.

## INTRODUCTION

Critical limb ischemia (CLI) is a pathological condition characterized by persistent ischemic pain and/or tissue loss (ischemic ulcerations and gangrene). CLI occurs when arterial lesions of distal limb extremities become severe enough to prevent and/or reduce effective blood flow and nutrition of distal tissues. Although CLI is associated with symptoms herein reported, the clinical diagnostic criteria of CLI are based on absolute ankle brachial index (ABI < 0.4), absolute ankle pressure (< 50 mmHg) and high toe pressure (< 30 mmHg). CLI generally involves a chronic degeneration of vessels related to the severe state of peripheral artery disease (PAD), which represents the clinical endpoint of PAD [1]. Several risk factors, in particular occlusive atherosclerosis, have been correlated to the development and progression of CLI. CLI can also be the result of atheroembolic or thromboembolic disease, vasculitis, *in situ* thrombosis related to hypercoagulable states, thromboangiitis obliterans, cystic adventitial disease, popliteal entrapment, or trauma. In fact,

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PAD compensatory mechanisms, such as distal vasodilatation and collateral formation, missed or decreased in patients with CLI [2]. Fontaine classified CLI as severe PAD damage that arises in patients with stages III-IV [3] or stages 4-6 (Rutherford classification) [4]. 1% of patients affected by CLI presents PAD damage and showed an overall mortality almost 50% at 5 years and 70% at 10 years [5]. The CLI morbidity in patients depends on race, sex and aging. Men are generally more affected then women (1.5:1) with a major amputation of limb in 45% of CLI patients after 6-12 months from early diagnosis [6].

The therapeutic strategy for CLI treatment depends on the severity of the disease and should be chosen as a function of the vascular damage. Macro-vascular modifications, as well as, micro-vascular dysfunction, are imposed as the primary goal of CLI treatment to alleviate ischemic pain, to heal ischemic ulcers, to prevent limb loss, to improve patient function and quality of life, and finally to prolong patient survival overall. In this attempt, the first-line therapeutic approach consists of surgery or limb revascularization through bypass and/or endovascular techniques, which represent the best option to prevent amputation and avoid physical impairment. To date, the US Food and Drug Administration has not approved any pharmacological therapy for CLI management [6].

Vasoactive drugs, in particular prostanoids, are actually the front line for CLI management. Prostaglandin $E_1$ ($PGE_1$) and its synthetic analogs (Iloprost or Endoprost) show *in vivo* platelet inhibition and leukocyte activation and promote vascular enlargement, thus improving local blood flow [7]. The systemic administration of $PGE_1$ in short (3-4 days) and long-term treatments (7-28 days) showed therapeutic benefits on reducing cutaneous ulceration and ischemic pain, but no suitable effects on vascular critical clinical symptoms. Furthermore, its therapeutic efficacy was limited to a few patients [2, 8, 9].

Recently, a review of 20 different randomized controlled trials comparing parenteral administration of $PGE_1$ versus placebo demonstrated that prostanoids efficiently inhibit pain (risk ratio (RR) 1.32, 95% confidence interval (CI) 1.10, 1.57) and ulcer healing (RR 1.54, 95% CI 1.22, 1.96), but there is no statistically significant correlation between the decrease of amputation and mortality rate in patients with CLI. The American College of Cardiology and American Heart Association (ACC/AHA) treated systemic administration of $PGE_1$ in CLI management as class IIb recommendations based on level A evidence, effect and safety of therapy. Iloprost administered as oral or systemic formulations shows a significant decrease of side effects compared to other $PGE_1$ injected systemically [8].

Physical-chemical properties of $PGE_1$ compounds can affect their stability in biological fluids. Although, therapeutic efficacy and side effects of $PGE_1$ are not affected by its chemical structure, Iloprost infusion still remains the main pharmacological treatment for CLI in patients that are not eligible for surgical or endovascular therapy [10]. The therapeutic treatment schedule for Iloprost infusion (0.5 and 2.0 ng/kg/min) is over 6 hours for 14-28 days [8]. The rapid systemic clearance (half-life decreased 30 min after infusion), hospital management and high cost of therapy limits its

clinical use. These drawbacks require the development of a suitable device providing constant plasma concentration and drug efficacy of Iloprost after systemic injection. Disposable infusion pumps (DPIs) have been widely used for several clinical applications, such as tumor treatment, antimicrobial therapy, pediatric applications, post-operative and chronic pain management.

Today, DIPs are compliant in patients compared with other infusion devices. In fact, they are easy to use, guarantee a constant and controlled release of filled drugs or formulations and allow domestic therapy without restricting daily activities [11]. DIPs with drug reservoirs from 60 to 500 ml have an infusion rate from 0.5 to 500 ml/h and release a constant volume of drug every 30 min for 12 days. Various devices show a fluid flow-rate that depends on the elastomer property (stretching of polymer under flow pressure) and present an outer protective sheath of soft elastomer or rigid plastic which surrounds the elastomeric membrane filled with drugs or formulations. Natural or synthetic polymers, as single or multiple layers, form walls of elastomeric membranes [12]. Drugs or formulations loaded into DIPs could potentially generate modifications of DIP structure, thus altering the integrity of membranes or generating drug metabolites. Drugs could aggregate and precipitate inside the disposable pump producing instability. In this attempt, the long-term stability of drugs or formulations inside DIPs, as solutions or suspensions, could represent a big challenge for CLI treatment with elastomeric pumps. In fact, aggregates or contaminants can block elastomers, hampering the diffusion of drugs or formulations, and could deliver nano-aggregates that further block the compromised vessels. These drawbacks can compromise the efficacy of therapy and exasperate risks in CLI treatment [13-15].

Moreover, salt solutions that dissolve drugs or formulations can modify their chemical structures thus compromising their folding and biological activity [16, 17]. The long term-stability of drugs and formulations dissolved in salt solutions keep contact with DIPs and can be monitored following different procedures, *i.e.* the measurement of pH modifications, size modification and distribution of particulates potentially contaminating formulations, conductivity, net surface charge and electrophoretic mobility of suspensions [18, 19]. However, some conventional methods cited above are time-consuming and invasive. Turbiscan technology can be useful and offer a valid solution to overcome these analytical drawbacks. In fact, Turbiscan Lab Expert has the advantages of shortening the time of analysis and preserves samples from degradation by being used more frequently in pharmaceutical sciences [20-24], cosmeceutics [25, 26], petroleum [27-30] and foods [31-33] to predict the long term-stability of inorganic and organic nano-materials and formulations.

The aim of this study was the evaluation of the long-term stability of Iloprost solution in an elastomeric DIPs using Turbiscan. In addition, Iloprost solution was evaluated in terms of constant and continuous release from DIPs over 24 h for 8 days. This therapeutic schedule based on patients affected by CLI has been selected to demonstrate the suitability of combination between Iloprost solution and DIPs, the improvement of patient compliance and the reduction of overall cost of treatment.

## MATERIALS AND METHODS

### Materials

Iloprost (5-{(*E*)-(1*S*,5*S*,6*R*,7*R*)-7-hydroxy-6[(*E*)-(3*S*,4*RS*)-3-hydroxy-4-methyl-1-octen-6-inyl]-bi-cyclo[3.3.0]octan-3-ylidene}pentanoic acid) solution (Endoprost 50 μg/0.5 ml) was a kind gift from Italfarmaco S.p.A., (Milan, Italy). Isotonic saline solution (NaCl 0.9 w/v) was purchased from Select High Srl, St. John Lupatolo (VR), Italy. The elastomeric infusion Vessel Fuser was purchased from HS Hospital Service S.p.A., (Aprilia, Italy).

### Iloprost Solution

Endoprost (8 vials, 50 μg/0.5 ml) was dissolved into 96 ml of isotonic saline solution and the final volume was adjusted to obtain 100 ml of formulation (4 μg/ml). The DIPs were filled through the Luer-lock connection system by using a syringe of 20 ml. DIPs were left at room temperature for 30 min to equilibrate solution before running the experiment. Iloprost solution-loaded DIPs were kept at room temperature for 8 days and a fixed volume (0.5 ml) was released from the elastomer at a flow rate of 0.5 ml/h.

### Turbiscan Apparatus

Turbiscan technique consists in sending photons (light) into the sample. These photons, after being scattered many times by objects in suspension (droplets, solid particles, gas bubbles) emerge from the sample and are detected by the device. A mobile reading head composed of a near infrared (NIR) diode and two detectors, *e.g.* transmission (T) and backscattering (BS), scans a glass cell containing the sample. The Turbiscan software then enables to interpret the obtained data easily. The measurement enables the quantification of several parameters, as BS and T values are linked to the average diameter (d) and volume fraction of the particles (φ). Turbiscan analyzes low and high concentration dispersions of Multiple Light Scattering in both T and BS mode.

T and BS signals depend on particle size (1 nm to 1 mm) and concentration range (0.0001 to 95% v/v) according to the following equation:

$$BS \ \& \ T = f\,(d/\varphi)$$

Turbiscan acquires T and BS every 20 microns along the sample height, thanks to a patented scanning reading head. Scans are repeated during sample ageing time to detect any variation of the signal due to a destabilization, such as particle migration and/or particle size variation. For more information see the following link: http://www.formulaction.com/stability-turbiscan-lab.html.

### Stability Analysis

The long-term stability of Iloprost solution was evaluated using Turbiscan Lab Expert (Formulaction, L'Union, France) [34]. Transmittance (ΔT) and backscattering (ΔBS) variations were carried out using a pulsed, near infrared LED at a wavelength of 880 nm. Samples were loaded into cylinder glass tubes and analyzed for 1 h for the entire length of the holder. The sample height (~ 20 mm) was scanned through two different synchronous optical sensors receiving the light transmitted through and backscattered by the sample at an angle of 180° and 45° respect to the incident radiation, respectively.

The physical-chemical stability of Iloprost solution-loaded DIPs was further evaluated using a pH-meter (WTW InoLab 720, Amtsgericht München, Germany). Standards (phosphate buffer at pH 4.0; 7.0 and 14.0, respectively) were used to calibrate the instrument before analysis. Replicates are measurements of six different samples ± standard deviation.

### Physical-chemical Characterization of Iloprost Solution

Conductivity and net surface charge and electrophoresis mobility of Iloprost solution were carried out using Zetasizer Nano ZS (Malvern Instruments Ltd., Worchestershire, UK) [35, 36]. All parameters were performed according to Smoluchowsky constant F (Ka) of 1.5. The apparatus was equipped with a He/Ne laser Doppler anemometry (633 nm) with a nominal powder of 5.0 mW. Samples were suitably diluted with isotonic saline solution (NaCl 0.9% w/v) before analysis to avoid multiscattering phenomena. Replicates are measurements of six different samples ± standard deviation. Isotonic saline solution was used as a control during experiments.

### High Performance Liquid Chromatography (HPLC) Analysis of Iloprost Solution

Iloprost solution was carried out using HPLC apparatus (Varian Inc., Palo Alto, USA) equipped with 200-2031 Metachem online degasser, a M210 binary pump, a ProStart 410 auto sampler, a G1316A thermostatic column, a 25 μl CSL20 Cheminert Sample Loop injector. Data was acquired and processed using Galaxie chromatography manager software (Varian Inc., Palo Alto, USA). A GraceSmart RP C18 column (4.6 × 250 mm, 5 μm, Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) was used as a stationary phase during analysis. Deionized water (HPLC grade) acidified with 0.1% v/v trifluoroacetic acid (TFA, HPLC grade) and acetonitrile (HPLC grade) 60:40% v/v was used as the mobile phase. The flow rate was 1 ml/min; while, Iloprost solution was acquired at a maximum wavelength of 205 nm. The drug was separated as an enantiomer at the retention times of 20.5 and 22 min, respectively. Enantiomers were widely separated during analysis and no interference peaks or overlapping of compounds were observed (Supplementary Fig. **1**). An external standard curve was carried out to quantify Iloprost solution according to the following equation:

$$AUC = 0.5899x + 0.0234$$

where, *x* is the Iloprost concentration (μg/ml) and *AUC* (mAU × min) is the area under the curve. A linear correlation with $R^2 = 0.9998$ was carried out in the range from 0.01 to 10 μg/ml.

### Statistical Analysis

Statistical analysis was carried out using one-way ANOVA. A posteriori Bonferroni *t* test was carried out to check the ANOVA test. A *p* value ≤ 0.05 was considered statistically significant. All samples are the average of at least three different experiments ± standard deviations.
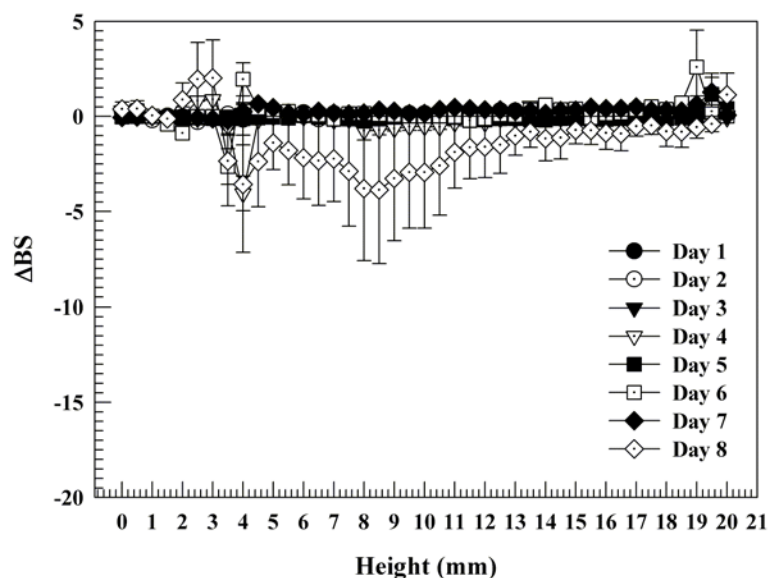
**Fig. (1).** Backscattering (ΔBS) profiles of Iloprost solution-loaded DIPs at different incubation times (0-8 days). Analysis was performed using Turbiscan Lab Expert and data has been analyzed using Turbiscan EasySoft Converter. Analysis was carried out for 1 h and samples were scanned from 0 to 20 mm in height. Results are the average of different measurements ± standard deviation.

## RESULTS

The long-term stability of Iloprost solution-loaded elastomeric DIPs was carried out using Turbiscan technology. It predicts the long-term stability of solutions or suspensions and represents a featuring tool in analytical methods. ΔBS and ΔT signals were carried out during analysis. These parameters allowed predicting the stability of Iloprost solution-loaded elastomeric DIPs through Turbiscan technology. Formulations were considered unstable for modifications of ΔBS and ΔT signals greater than 10% either as positive or negative values on a graphical scale. Turbiscan data were further supported by HPLC analysis, pH and dynamic light scattering (DLS) measurements.

### Stability of Iloprost-loaded Elastomeric DIPs Through Turbiscan Technology

ΔBS and ΔT profiles of Iloprost solution-loaded DIPs demonstrated that no significant modifications of both signals occurred during analysis. Samples (~20 mm) were scanned for 1 h and the modification of ΔBS signals was lower than ± 0.5% and could be overlapped with baseline (Fig. **1** and Supplementary Figs. **2** and **3**). ΔBS analysis at different incubation times (from 5 to 8 days) also demonstrated that the Iloprost solution maintains its stability into DIPs and the long contact between the drug solution and the polymeric materials of DIPs did not modify the chemical stability of the formulations (Supplementary Fig. **2**). Furthermore, particles were not released from the elastomeric pump and no variation of particle size was observed in the sample solution according to the dynamic light scattering analysis.

The long-term stability of Iloprost solution-loaded DIPs may be influenced by formulation excipients, *e.g.* 96% v/v ethanol. The dilution of Endoprost with isotonic saline (NaCl 0.9% w/v) elicited no instability in the final solution and the presence of ethanol determined no polymer release from the walls of DIPs. Furthermore, the long contact between the drug solution and polymers determined no physical-chemical modification of both the solution and the DIPs structure (Supplementary Figs. **2** and **3**). The ΔT analysis showed similar profiles with regard to ΔBS (data not shown).

The long-term stability of Iloprost solution-loaded DIPs was further demonstrated by analyzing the slope of ΔBS at different incubation times (from 1 to 8 days). Results demonstrated that the slope of ΔBS for Iloprost solution at short (from 1 to 4 days) and long (from 5 to 8 days) incubation times was not significantly modified during analysis. The slope values ranged from 0.01 and 0.02 (Figs. **2** and **3**).

### Stability of Iloprost-loaded Elastomeric DIPs Using HPLC Analysis

The HPLC chromatograms at different incubation times (from 1 to 8 days) showed the same retention times (20.2 and 21.5 min) obtained by injecting Iloprost solution, which was used as the standard during analysis (Supplementary Fig. **1**). The shape of the peaks was not modified for both enantiomers at different incubation times (Supplementary Figs. **4** and **5**). HPLC chromatograms also showed that both enantiomers were suitably separated. HPLC data agreed with Turbiscan analysis.

### Release of Iloprost Solution from Elastomeric DIPs

The DIPs had a constant (Supplementary Table **S1**) and continuous release (Fig. **4**) of Iloprost solution from day 1 to day 8, thus it did not modify the therapeutic drug dosage during incubation times. The daily therapeutic release of Iloprost solution was maintained constant after loading into DIPs (Supplementary Table **S1** and Fig. **4**).

### pH Measurements of Iloprost-loaded Elastomeric DIPs

The measurement of pH solution before and after loading the drug solution into DIPs can be used as a suitable indicator
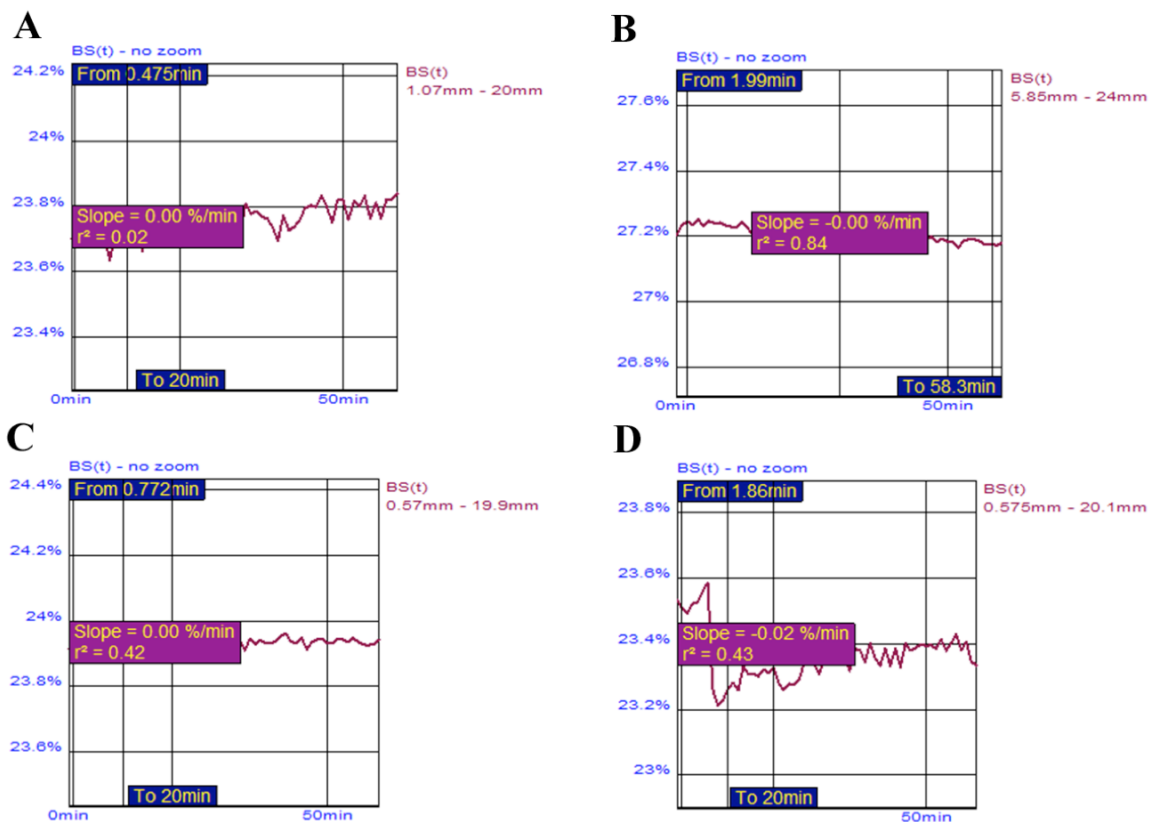
**Fig. (2).** Kinetic profile of backscattering ($\Delta$BS) for Iloprost solution-loaded DIPs. The slope of $\Delta$BS was evaluated at different incubation times: (**A**) day 1; (**B**) day 2; (**C**) day 3; (**D**) day 4. 20 mm of samples were analyzed for 1 h at room temperature. Results are the average of three different measurements ± standard deviation.
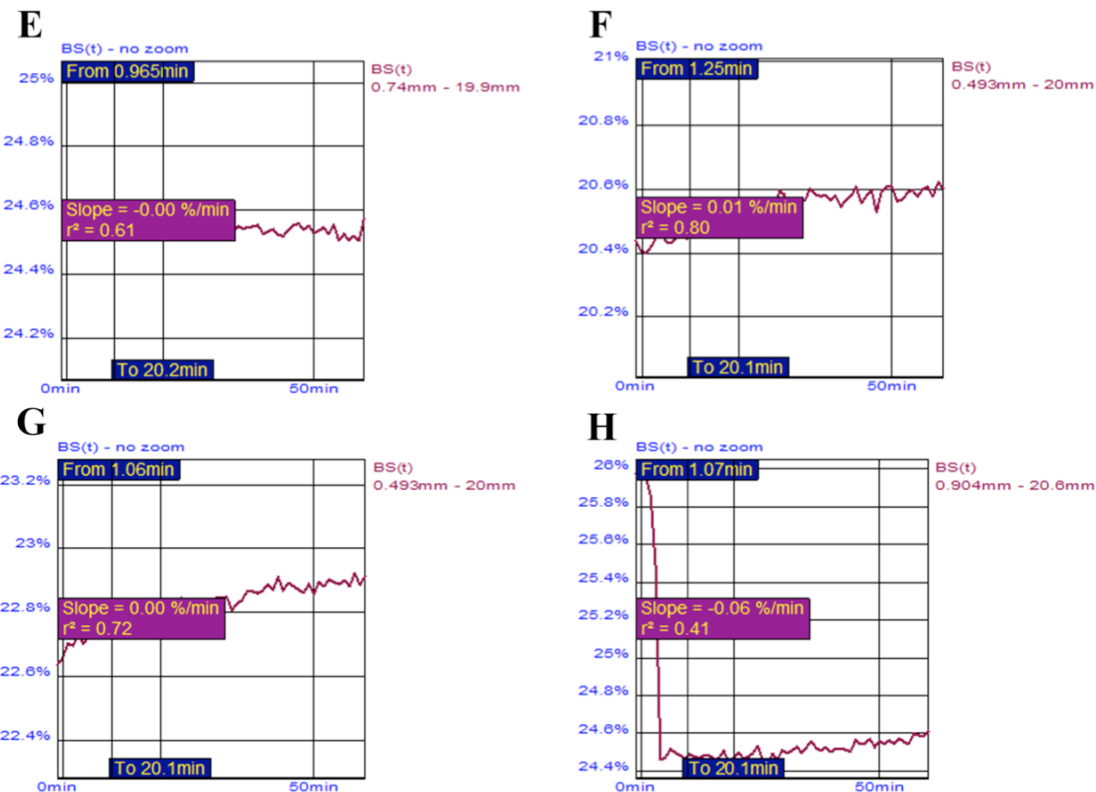


**Fig. (3).** Kinetic profile of backscattering ($\Delta$BS) for Iloprost solution-loaded DIPs. The slope of $\Delta$BS was evaluated at different incubation times: (E) day 5; (F) day 6; (G) day 7; (H) day 8. 20 mm of samples were analyzed for 1 h at room temperature. Results are the average of three different measurements ± standard deviation.
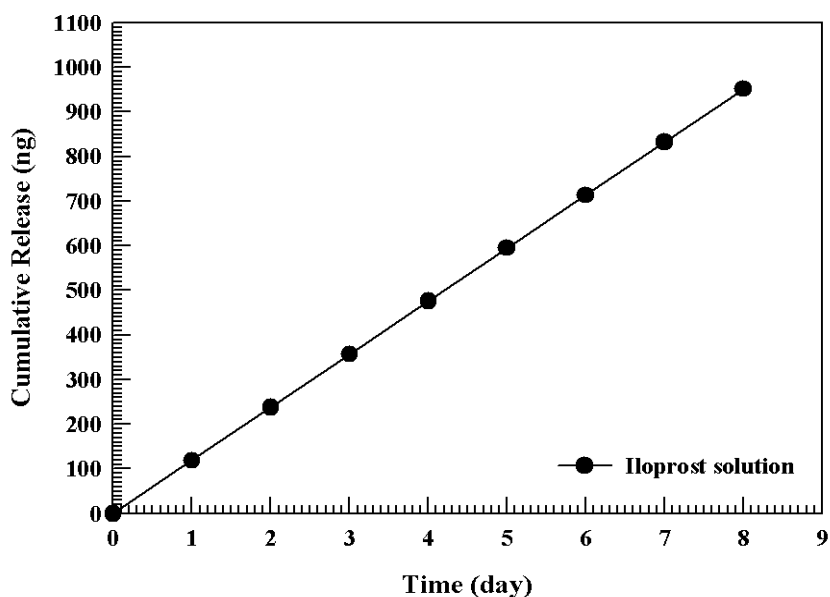
**Fig. (4).** Cumulative release of Iloprost solution from DIPs. The release profile was performed until 8 day. Iloprost solution was prepared and loaded into DIPs as reported in Materials and Methods section. The quantification of drug was carried using HPLC. Results are the average of three different measurements ± standard deviation.
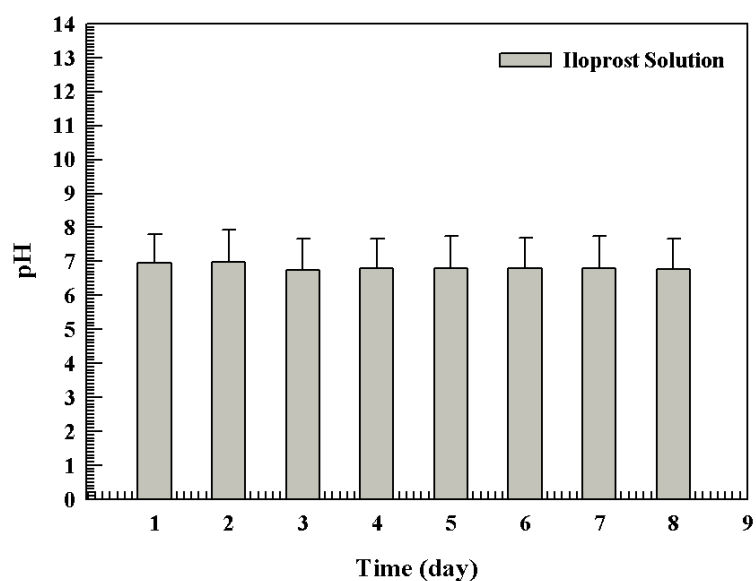


**Fig. (5).** pH modification of Iloprost solution-loaded DIPs. pH was monitored at different incubation times (0-8 days). Results are the average of three different measurements ± standard deviation.

to further evaluate the stability and compatibility of the Iloprost solution in the elastomeric pump. The pH measurements might allow providing suitable information about the physical stability of the drugs; for this reason, the change of pH was tested to measure the stability of Iloprost solution loading DIPs. Results demonstrated that the pH was not modified for Iloprost solution loaded inside DIPs, thus not affecting its stability. Conversely, pH modification might modify the chemical structure of Iloprost and its stability in fluids.

The pH of Iloprost solution-loaded DIPs was monitored over time. No significant modification of pH was observed as a function of incubation times (from 1 to 8 days) compared to Iloprost standard solution and a pH value of 7.0 was

observed (Fig. **5**). The data demonstrated that Iloprost solution is stable in DIPs and the long contact between the drug and the elastomeric pump does not affect its physical-chemical stability. These results agree with HPLC data (Supplementary Figs. **4** and **5**) and Turbiscan analysis (Supplementary Figs. **3** and **4**).

**DLS Measurements of Iloprost-loaded Elastomeric DIPs**

The physical-chemical stability of Iloprost solution-loaded DIPs was further evaluated by measuring conductivity, net negative charge and electrophoresis mobility. Results demonstrate that conductivity is not modified in Iloprost solution maintaining prolonged contact with elastomeric pump. 20 mS/cm was measured for Iloprost solution at dif-

ferent incubation times (from 1 to 8 days), and these values are not statistically different from those observed in the case of NaCl (0.9% w/v) isotonic solution (19.80 mS/cm) (Supplementary Table **S1**). Constant values of conductivity also demonstrated that Iloprost was not modified being kept up to 8 days at room temperature.

The measurement of Iloprost solution charge further demonstrated that it maintains a net negative charge at physiological pH (7.4), values ranged from -7.31 to -15.20 mV (Supplementary Table **S1**). The net negative charge of Iloprost solution at NaCl 0.9% w/v isotonic solution could depend on the hydration of the drug molecules and hydrodynamic equilibrium occurring between pH solution and the functional group of Iloprost. Furthermore, the net negative charge measured for different samples are higher than isotonic physiological solution (-1.710 mV) used as control during the experiments (Supplementary Table **S1**). Table **S1** demonstrates that the net negative charge of Iloprost solution increased over -7 mV after 6 days. The electrophoretic mobility of the samples agreed with the net negative charge and no statistically significant difference was observed as a function of incubation time.

## DISCUSSION

The Turbiscan technology represents a useful tool to predict the long-term stability of formulations which dissolve different drugs and excipients. The physical-chemical principle of Turbiscan analysis assesses the stability of solutions and suspensions by measuring the particle variation of the droplet volume fraction (migration) or mean size (coalescence). This method enables faster detection than conventional methods by detecting instability phenomena at an early stage. Results are shown as modifications of $\Delta BS$ and $\Delta T$ signals. Positive (backscattering increase) or negative peaks (backscattering decrease) may occur during analyses of different solutions or suspensions, and the migration of particles from the bottom to the top and *vice versa* can be correlated to instability phenomena occurring in formulations [21, 34]. The Turbiscan technology also predicts the long-term stability of solutions or suspensions by avoiding manipulation, dilution or disruption of samples [37, 38].

Variations in the transmission and/or backscattering profiles of samples within an interval of $\pm$ 2% are not considered significant, thus demonstrating no variation and/or migration of particles during the analysis, namely the case of stable dispersed formulations. Formulations were considered unstable for modifications of $\Delta BS$ and $\Delta T$ signals greater than 10% either as positive or negative values on a graphical scale. Turbiscan technology also predicts the long-term stability of solutions or suspensions by avoiding manipulation, dilution or disruption of samples [37, 38]. Iloprost solution-loaded DIPs demonstrated that $\Delta BS$ and $\Delta T$ profiles were not modified during incubation times, and drugs, as well as, DIPs do not change their composition, chemical structure, shape and rearrangement during the analysis. $\Delta BS$ signals were lower than $\pm$ 0.5% and could be overlapped with baseline as previously reported for colloidal liposomal formulations [20, 21, 34]. Results demonstrated that formulations are stable during the incubation times, even with the presence of

high peaks at the beginning of Turbiscan analysis. This peak did not show the presence of instability phenomena of the samples but it could be related to air-liquid droplets occurring at the interfaces of the samples on the top and bottom of the glass cylinder [38].

The excipient included in Endoprost may affect the long-term stability of drugs-loaded DIPs. In fact, ethanol (96% v/v) added to Iloprost solution may improve its water solubility and avoid the detachment of polymers from DIPs without modifying the structure of elastomeric pump (Supplementary Figs. **2** and **3**). These findings also demonstrate that Iloprost solution could be compatible with DIP devices, thus answering the high compatibility requirement for DIPs, whose plastic parts remain in contact with the drug formulation for a relatively long time [12].

The long-term stability of Iloprost solution-loaded DIPs and its release from elastomeric pump were further performed using HPLC method. HPLC chromatograms demonstrated that the shape and the height of peaks were not changed under the incubation times. Furthermore, both enantiomers forming Iloprost standard solution, as well as the compounds released from DIPs, were suitably separated (Supplementary Fig. **1**).

The rapid decrease of drug concentration during the first 24 h of contact with the disposable pump shows that the drug is absorbed in the container walls, and unstable phenomena may occur by contact between the drug solution and DIPs [12]. The measurement of pH solution before and after loading the drug solution into DIPs can be used as a suitable indicator to further predict the stability and compatibility of the Iloprost solution in the elastomeric pump. In fact, pH modifications may be elicited by the drug degradation or precipitation inside DIPs [12]. The pH modification of the drug solution in the elastomeric pump can produce color change, precipitation, weigh/water loss of the drug solution, as well as plasticizer and degradation products inside the solution [39]. Iloprost was diluted to obtain a suitable drug therapeutic concentration before loading inside DIPs. In fact, a technical report showed that DIPs have specific diameters and lengths for mechanical flow restriction and flow rate depending on volume, viscosity, and pressure gradient across flow restrictor and pressure (250-600 mmHg). The dilution of the drug through an isotonic solution does not generally provide the pH modification of $PGE_1$, particularly Iloprost. pH was used as a suitable parameter to evaluate physical stability of the drugs; for this reason, the change of pH was tested to measure the stability of Iloprost solution loading DIPs. Results demonstrate that the pH is not modified for Iloprost solution loaded inside DIPs, thus not affecting its stability. Conversely, pH modification may modify the chemical structure of Iloprost and its stability in fluids.

The pH of Iloprost solution-loaded DIPs was monitored during the time and no significant modification of pH was observed as a function of incubation times (from 1 to 8 days) compared to Iloprost standard solution. pH measurements agreed with HPLC and Turbiscan analysis demonstrating that this technology could be used as a novel tool to predict the long-term stability of formulation-loaded DIPs.

To enforce the potential application of Turbiscan technology, the physical-chemical stability of Iloprost solution-loaded DIPs was further evaluated by measuring conductivity, net negative charge and electrophoretic mobility through DLS. Results demonstrated that Iloprost solution-loaded DIPs still remain stable for a long time and Iloprost solution was not drastically modified being kept up to 8 days at room temperature. Furthermore, these results evidenced a good compatibility and stability of Iloprost solution with the elastomeric component of DIPs [12].

A net negative charge of Iloprost solution occurred at physiological condition (pH 7.4) and in the presence of isotonic solution. This net negative charge may depend on the potential hydration of compounds and hydrodynamic equilibrium occurring between pH solution and the functional group of Iloprost in solution. Furthermore, any modification of electrophoretic mobility and net negative charge of Iloprost solution might be attributed to the adsorption of the drug or its metabolites on DIP surface during the incubation times [40, 41].

## CONCLUSION

Turbiscan technology can be used as a suitable and non-time consuming analytical method to predict the long-term physical-chemical stability of suspensions of colloidal formulations. This is a non-invasive and rapid method to investigate the long-term stability of colloidal formulations and dispersions containing surfactants, preservers and different agents, which can potentially modify the stability of native products. Turbiscan technology can provide suitable information about the potential stability of pharmaceutical formulations, parenteral emulsions, antibiotics and therapeutic drugs confined inside DIPs, such as Iloprost solution. Results demonstrated that Iloprost solution does not modify its physical-chemical features, still maintaining a long-time contact with elastomeric pumps. pH, conductivity and electrophoretic mobility are not modified over time of incubation and data can be overlapped with isotonic physiological solution used as control. The elastomeric pump further demonstrated a constant and continuous release of Iloprost solution from DIPs and could be eligible as suitable biomedical devices for domestic treatment of CLI. This therapeutic treatment of patients affected by CLI could improve patient compliance and efficaciously decrease the costs of hospitalization.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest or financial interests in any products or service mentioned in this article, including grants, employment, gifts, stock holding, or honoraria.

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## SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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