Revista CENIC Ciencias Químicas, Vol. 36, No. Especial, 2005

High quality water production for pharmaceutical use by Ozone treatment.

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ABSTRACT. The pharmaceutical industry is a great consumer of high quality water, which must fulfill severe specifications concerning chemical impurities and endotoxins. In the present work, an industrial installation was built and operation parameters were searched for a high quality water production process by ozone refining treatment. In the design and building of ozonation system, and also in the techniques and procedures for the quality control assurance of the process and the final product, GMP, GLP and up-to-date Pharmacopeia were taken into account. Tap water previously treated to obtain purified water with suitable values of initial conductivity was employed for ozonation. Different ozone concentrations in the gas and in the liquid, liquid flows and the effect of ultraviolet light were assayed. A flow diagram description of the ozonation process is presented. A challenge to the system was conducted in which endotoxins were inoculated into the purified water. The effect of ozonation treatment on endotoxin content was determined. It was shown that, starting with water with good initial conductivity and by an ozonation polishing process it is possible to industrially produce high quality water for pharmaceutical use, which fulfills the international standards. A preliminary analysis showed the economic feasibility of this process compared to another where distillation is employed as a final step.

RESUMEN. La industria farmacéutica consume grandes cantidades de agua de alta calidad, que debe cumplir estrictas especificaciones en lo que concierne a impurezas químicas y endotoxinas. En el presente trabajo se construyó una instalación industrial y se investigaron los parámetros de operación para la producción de agua de alta calidad mediante un tratamiento de afinado con ozono. En el diseño y construcción del sistema de ozonización, así como en las técnicas y procedimientos utilizados para el control y aseguramiento de la calidad, se tuvieron en cuenta las Buenas Prácticas de Producción, las Buenas Prácticas de Laboratorio y las farmacopeas vigentes. Para la ozonización se empleó agua de acueducto, previamente tratada para obtener agua purificada con valores adecuados de conductividad inicial. Se ensayaron diferentes concentraciones de ozono en el gas y en el líquido, así como el efecto de la luz ultravioleta. Se presenta un diagrama de flujo del proceso de ozonización. Se realizó un reto al sistema mediante la inoculación de endotoxinas en el agua purificada. Se determinó el efecto de la ozonización sobre el contenido de endotoxinas. Se demostró que, partiendo de agua purificada con buena conductividad inicial, mediante un tratamiento de afinado con ozono es posible producir agua de alta calidad para uso farmacéutico, que cumpla con las normas internacionales. Un análisis preliminar muestra la factibilidad económica de este proceso comparado con el que utiliza la destilación como paso final de la purificación.

Key words: ozone; high quality water, water for injection, endotoxin degradation.

Palabras clave: ozono, agua de alta calidad, agua para inyección, degradación de endotoxinas.

INTRODUCTION

The pharmaceutical industry consumes great volumes of high quality water as water for process and/or in the production of medicaments. According to the United States Pharmacopeia (USP) 26[1], effective since 2003, there are two main kinds of high quality water for pharmaceutical uses: purified water (PW) and water for injection (WFI). These waters must fulfill strict standards concerning chemical and microbiological impurities.
Whereas the USP 26 monograph states that PW can be produced by any suitable process, for example distillation, reverse osmosis (RO) or ion-exchange treatment, WFI being the highest quality water in the

pharmaceutical industry, which must meet the most severe quality standards established in the USP, (conductivity, TOC and endotoxin values) should be obtained by distillation or reverse osmosis.
Nevertheless Pharmacopeias from other countries (for example British Pharmacopeia BP 2000)[2] only accept distillation as a method to obtain WFI. This restriction is based in the fact that distillation, although a great energy consumer, is the safest accepted method of purifying water, and because in the past it has been shown that sometimes RO was not capable to steadily control conductivity or endotoxin levels within required values for WFI and there were not enough reliable validation studies. In practice some of pharmaceutical industries combine RO and multistage distillation to produce WFI, with a rather energy high consumption.
Ozone is a well-known disinfectant that inactivates bacteria, fungi, viruses and parasites. Scientific, technological and patent literature on this subject is abundant. Ozone is also a very powerful oxidant and readily oxidizes many organic substances in aqueous solution, but usually ozone by itself does not convert them to water and carbon dioxide, so complete removal of TOC is seldom attained. It has also been reported that ozone, alone or in combination with ultraviolet radiation can readily destroy endotoxins.[3,4]
In the pharmaceutical industry ozone has been employed for loop sanitation, sterilizing water and cleaning and sterilizing containers. However the literature related to the possibility of obtaining high quality water, which fulfills WFI standards by ozonation treatment, is scarce.[5,6]
Taking into account that in general ozone treatment does not diminish the conductivity of water it was thought that starting with a PW that fulfills this parameter requirement for WFI, it could be possible through a polishing ozone treatment to industrially produce high quality water, which met USP 26 standards for WFI, in a safe and steady way Therefore the present work was carried out with the following purposes:

- Design, setting up and start up of an industrial ozonation system to obtain high quality water for pharmaceutical uses.
- To study the influence of some operation conditions on the quality of ozonized water.

MATERIALS AND METHODS

Experiments were carried out at a pharmaceutical industry, which produces parenteral drugs in Havana, Cuba.
PW there obtained according to GMP was used as feed water for ozonation treatment system. Fig. 1 shows a flow diagram of the present PW system employed in that factory. Basically, tap water is firstly chlorinated, filtered and softened, filtered through granular active carbon (GAC) and passed by a two-pass reverse osmosis (RO). A solution of sodium hydroxide was pumped at a point before the RO first step. A pH sensor placed online in between the two-pass RO automatically controlled addition of sodium hydroxide.

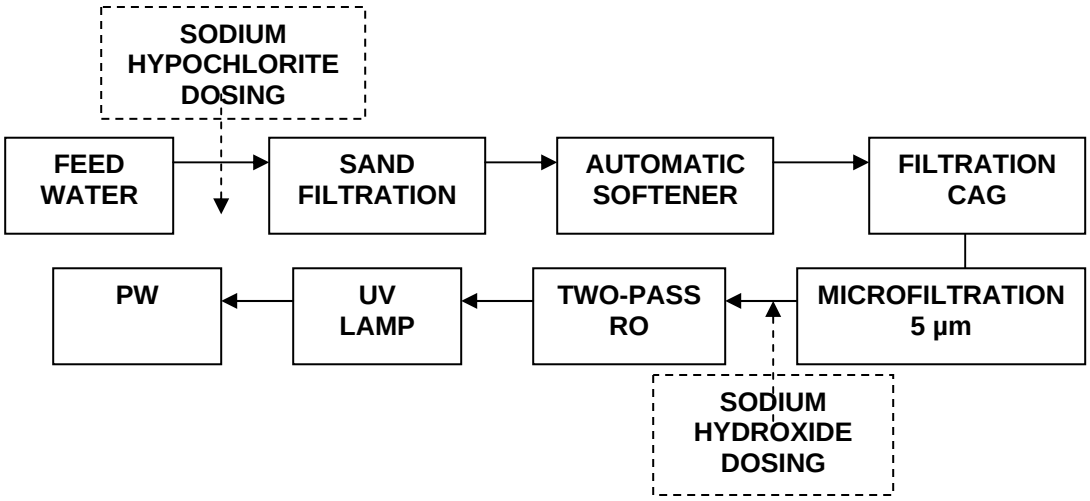

Fig. 1. Flow diagram for PW production

Ozonation system design and setting up

Ozonation system was designed and built in order to meet GMP. All the components of the ozone treatment system, including tank, pump, valves, column, pipes, clamps and fittings were made of stainless steel 316L with roughness below 0.5 μm, certified for sanitary applications and controlled in origin places. Attention was paid in avoiding dead-legs as much as possible and in enabling suitable piping slope and water flow rates to preclude favorable conditions for microorganism growth.

All welding work were carried out by automatic orbital welding under high purity argon atmosphere and performed by accredited skilled welders. Quality of resulting welds was determined by X-ray examination. Prior to system use, inner surface of system components was chemically passivated with citric acid.

Ozonation system

A schematic diagram of the ozonation system employed is shown in Fig. 2. Water from recirculating PW loop entered at the top of a 300 L bubbling column. Ozone, generated from oxygen in an ozone generator OZC1 (Trailigaz, France) was introduced at the bottom of the column through porous diffusers so water was continuously ozonized with countercurrent contact between gas and liquid phases. An ultraviolet lamp (Wedeco, Germany) was placed between column and tank, from where a pump (Alfa Laval, Sweden) recirculated water through the loop to point of use and column. In line UV lamp was used at point of use. Optionally water could be independently recirculated to tank and/or to column.

The system has sampling ports at the inlet an outlet of the column, and also after the UV lamp. Residual ozone gas was catalytically destroyed. Vent filter were coupled at exhaust gas exit. Ozonation system was provided with an automatic control that stopped system operation in case of malfunction.

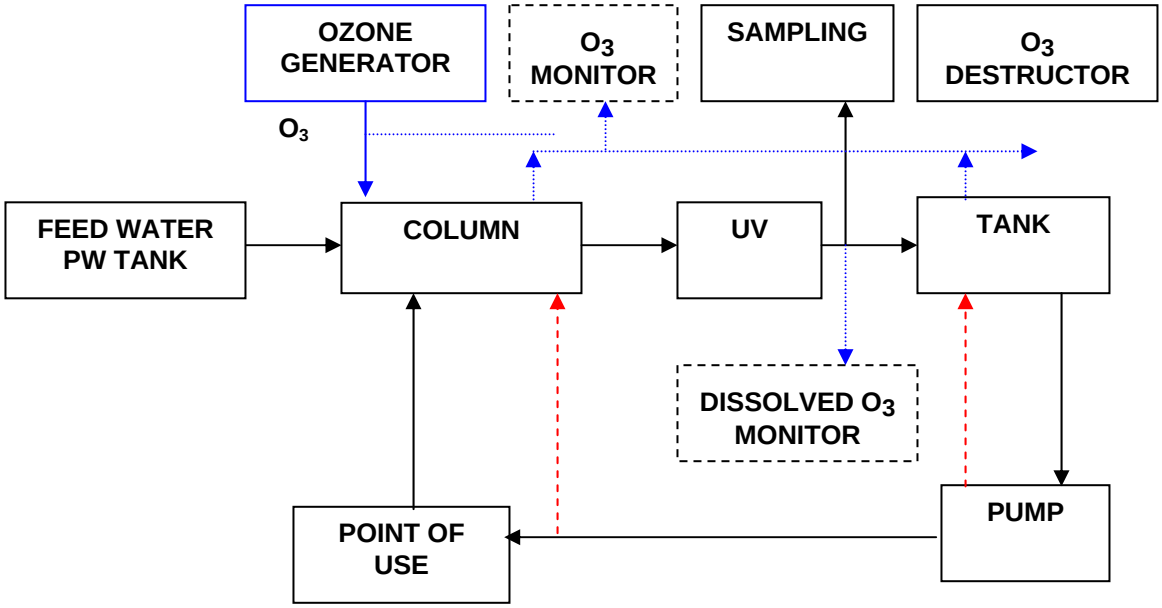

Fig. 2. Ozonation process flow diagram.

Ozonation operation conditions and parameter control

Experimental conditions were fixed at the following values:

- Inlet gas ozone concentrations: 40 and 80 mg/L
- Water flow: 400 and 600 L/h
- Gas flow: 500 L/h.
- In the study some experiments were accomplished with the UV lamp switched on.

Inlet/outlet ozone concentrations in the column were measured with an Anseros analyzer (Germany). Dissolved ozone concentrations were measured in a Dulcometer monitor (Germany) and by indigo trisulfonate method [7] modified by Gordon et al.[8]

Quality controls to PW and ozonized water were accomplished following validated standard operation procedures (SOPs) according to USP 26 and GLP, as established in the factory. In each experimental run, conductivity, TOC and endotoxin level were measured in ozonized water. TOC analysis was carried out in a

TOC-5050 analyzer from Shimadzu (Japan). Endotoxin was quantified by chromogenic Limulus Amebocyte Lysate (LAL) test.

Experiments with endotoxins

E. coli strain ATCC 10536 was inoculated from a nutrient agar plate into 10 mL of tryptone soy broth and grown for 18 hours at 35 ^{0}C. A loopful of this culture was subcultured again into 10 mL of tryptone soy broth and grown for 18 hours at 35 ^{0}C. The complete volume of culture was centrifuge for 5 minutes at 6500 rpm. Resulting pellet was submitted to ethanol (70%) during 1 hour. Later it was centrifuged, washed two times and finally resuspended in 10 mL of sterile distilled water. A sonic cell destructor was employed to provoke cell lysis. Volumes of the resultant suspension were taken to adjust the initial endotoxin concentration in the desired range for each trial.

To carry out endotoxin challenge, the original arrangement of the system was slightly modified, as shown in fig. 3.

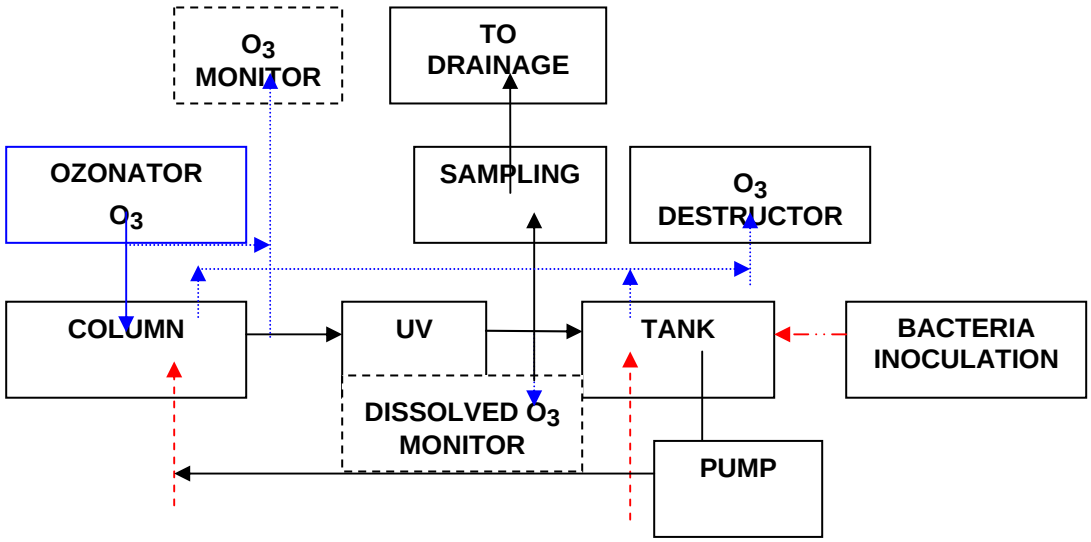

Fig. 3. Endotoxin challenge flow diagram

Once tank and column were filled with PW, a certain volume of bacteria suspension was introduced in the tank, and water was recirculated to homogenize endotoxin content in the whole system. Water flow was fitted to the desired value and conducted to drainage. At this time, ozonation started. Samples were taken out at different reaction times to perform control analysis.

RESULTS AND DISCUSSION

Effect of experimental variables on water quality

In a first study, the influence of several experimental conditions (ozone concentrations in the gas, ultraviolet irradiation and liquid flow) on the quality of final water was tried to establish.

Some experimental difficulties arose in the way to achieve this goal. Firstly conductivity of PW obtained after RO fluctuated above and below 1.3 μScm^{-1}, so in practice it did not fulfill all the time long the required USP 26 value, in spite of NaOH dosing to avoid carbon dioxide to pass the RO membranes. Therefore it was necessary to control that only PW with conductivity values below 1.3 μScm^{-1} passed to PW tank. In a second place under the experimental conditions employed, which were not exactly the normal operation ones, water temperature in the system was not controlled and continuously increased as a result of friction due to recirculating water flow, affecting the effective gas and dissolved ozone concentrations, which decayed in time.

Examples of the results obtained in this study are shown in Table 1.

Table 1: Examples of results obtained in feed PW and ozonized water

			THIS WORK	
PROPERTY	UNITS	WFI (USP 26)	FEED WATER	OZONIZED WATER
CONDUCTIVITY	μScm^{-1}	$\leq$ 1.3 ($25^{O}C$)	$\cong$1.0	0.8-1.1
TOC	ppb ($\mu g\ L^{-1}$)	$\leq$ 50O	462.7*	362.9*
ENDOTOXINS	E U mL^{-1}	$\leq$ 0.25	$<$ 0.25	0.211, 0.109, 0.091

* Mean values

As seen, resulting ozonized water met standard values for all of the parameters. In some experiments, ozonized water conductivity slightly increases (less than 0.1 unit) as a result of ozonation reaction, compared with feed water conductivity measured under the same conditions. In few of the runs feed PW TOC exceeded 500 ppb (maximum value 547 ppb).
Endotoxins levels for feed and ozonized water always matched accepted values.
During ozonation treatment TOC and endotoxin contents diminished. However although it was shown that ozonation treatment improved these values, since in most of the cases initial feed water also already fulfilled all the requirements for WFI, it was impossible to unequivocally determine how changes in experimental conditions influenced the quality of the final water obtained during ozonation treatment.

Endotoxin challenge

In a second trial, system was challenged by initially adding a bacterial suspension. These experiments were carried out aiming to establish whether, in case of casual contamination, ozonation process could sufficiently ameliorate endotoxin level till meeting USP 26 standards. As previously reported [4] that maximum endotoxin level expected for treated potable water could be in the range of 0.5 to 5.0 endotoxin units /milliliter (EU/mL), considering the purification operations used in obtaining the PW employed, endotoxin initial values were fixed between 0.25 and 1.0 EU/mL. In these experiments, after carrying out a preliminary experimental design, in which both O_3 concentrations were assayed, O_3 concentration of 40 mg/l in the gas phase was discarded, so since then only the higher ozone gas concentration of 80 mg/l and water flow of 400L/h were employed.

Table 2 shows examples of the results obtained.

Table 2: Examples of results obtained in feed PW and ozonized water after endotoxin challenge

	UNITS	CHALLENGED FEED WATER	OZONIZED WATER
ENDOTOXINS	E U mL^{-1}	0.70-0.51-0.31	0.11, $<$ 0.09

Results demonstrated that with the ozonation process employed, it is possible to obtain water that fulfills WFI standards even if starting endotoxin content is high within rational limits.

Cost analysis

Finally a preliminary comparative analysis with respect to investment and operating costs was done. Proposed ozonation process was compared with the present one used at the factory, which have in common all the unit operations shown in Fig. 1 but in which WFI is finally produced by a multistage distillation. In the analysis only basic equipment investment and operation costs related to the whole ozonation process and distillation operation were considered. Salaries and the rest of additional costs were not included.
From Table 3 it is obvious that proposed ozonation process is more economical than distillation. Both distillation investment and operation costs are considerable greater Advantages are not only in equipment investment, in which the cost of distillation still surpasses several times the costs of an ozonation system, but also in energy and maintenance savings.
A throughout validation of the installation and ozonation process will be soon in progress.

Table 3: Comparative estimated investment and annual operation costs*

Ozone treatment		**Distillation**	
Investment costs	**USD**	**Investment costs**	**USD**
Ozonator	12 000	Distillation still	**277 000**
Tank	6 400		
Column	2 800		
Pump	4 400		
UV Lamp (6 points of use)	4 600		
Electrodes	900		
Ozone Destructor	300		
Filter Cartridges	100		
TOTAL	**31500**		**277 000**
Operation Costs/Year		**Operation Costs/Year**	
Depreciation	3 150	Depreciation	27 700
Maintenance	650	Maintenance	5 500
Power Consumption 8 kWh x 4 800 h/year	3500	Distillator Energy Consumption** ❑ Estimated distillation still oil consumption 240 L/d	19 200
TOTAL	**7 300**	**TOTAL**	**52 400**

*** Calculation basis: 24h/day, 20 days/month, 10 months/year**
**** Oil equivalent**

CONCLUSIONS

- An industrial ozonation installation to obtain high quality water for pharmaceutical industry in compliance with GMP has been built.
- It has been demonstrated that, under the conditions studied, the proposed industrial polishing ozonation process can steadily, economically and safely produce water that fulfills the strictest standards for pharmaceutical uses even if starting endotoxin content is high within rational limits.

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