

Aqueous solutions in sealed containers: internal pressure under autoclaving

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ABSTRACT: This article is a technical support to the practice of autoclaving aqueous solutions in sealed containers. Another article of his, Autoclaving aqueous solutions in sealed containers: feasibility and risks, provides a more practical guide on the matter. Two “classical” scientific contributions on the matter are due to Beck and to M. A. Joyce & J. W. Lorenz and referred in the article. In this article, metric units (abs bar or abs mbar) are used for the pressure. 1 abs bar is approximately equivalent to 14.504 psia.

KEYWORDS: moist-heat sterilization, steam sterilization of aqueous solutions, sealed containers, headspace volume, internal pressure, autoclaving, autoclave, superheated water sterilizers, steam/ air mixture sterilizers

The pressure in the headspace of sealed containers filled with liquid *solutions* is the sum of:

- a) the pressure of the vapor in equilibrium with the liquid of the solution, i.e. vapor pressure of the liquid solution at the actual temperature;
- b) the actual pressure of the gases escaped from the liquid solution;
- c) the actual pressure of the gas initially filling the head space.

In the case of liquid mixtures of non-soluble components (i.e. of multiphase liquids), the addendum a) is to be replaced by the sum of all the vapor pressure addenda of the single components of the liquid itself. In spite of their pseudo-homogeneous aspect, emulsions are a typical case of multi-phase liquid: thus, their vapor pressure is the sum of the vapor pressure addenda of the single liquid phases at the actual temperature, regardless to the weight percentage of the single components of the liquid mixture.

The volume of headspace depends in turn on the temperature, due to the effect of thermal expansion of the liquid, that tends to reduce the head space, and to the opposite effect of the thermal expansion of the material by which the container is made, that tends to increase the head space.

An important question is: **how will the change of the headspace volume affect the total pressure in it under autoclaving?**

- a) **Vapor pressure.** The first out of the above pressure addenda depends

only the temperature: the vapor pressure of the liquid solution follows

the P *versus* T curve of the vapor and liquid equilibrium of the solution.

- b) **Escaping of dissolved gases.** The second addendum is a complex case: in fact, the pressure of the gases escaped from the liquid solution is proportional to the molar fraction of the gases dissolved in the liquid solution and to a coefficient that increases with the temperature. This proportionality coefficient is frequently referred to as “Henry’s constant”. It does not vary so much with the partial pressure of the gas: in the case of nitrogen and of oxygen, this change is about 10% if the partial pressure undergoes a ten-fold increase, and is almost constant at lower pressure values. With the temperature, the Henry constants for nitrogen and oxygen approximately double between ambient temperature and current sterilization temperatures: this corresponds to the fact that the solubility of gases in liquids decreases with the temperature. For another gas also used, even if seldom, as blanketing medium in pharmaceutical operations, i.e. the carbon dioxide, the changes of the Henry’s constant with the temperature and with the pressure of the gaseous phase are appreciably bigger.

For practical purposes, from the point of view of the pressure inside sealed containers under autoclaving, it may be assumed that about the half of the *nitrogen and oxygen* initially present in the solution will escape into the headspace at sterilization temperature. As Henry’s constants of nitrogen and oxygen are only poorly affected by the change of the partial pressure of the escaped gas, it may be expected that any reduction of the

headspace volume have the effect of re-dissolve again a certain amount of it, in order to restore the equilibrium. Thus, for practical purposes, it is possible to assume that the pressure made-up in the headspace by the initially dissolved nitrogen and oxygen depends only on the temperature of the liquid, but is independent of the changes of the head space volume.

For the *carbon dioxide*, the escaped fraction under autoclaving conditions could be higher than 80% of the initially dissolved amount, but the effect the pressure increase in the headspace for re-dissolving the escaped gas is bigger than in the case of nitrogen and oxygen. This entails that in the case of carbon dioxide any reduction of the headspace has also no dangerous effects. Nevertheless, it is important to note that the solubility of carbon dioxide in water is much higher than the solubility of the two major air components, so that the contribution of carbon dioxide to the pressure in the headspace can be forfeited only if it is present in very small quantities in the blanketing atmosphere. This occurs when the blanketing gas is air (or nitrogen or oxygen separately). On the contrary, if carbon dioxide itself is used as main blanketing gas, a detailed study of the case will be necessary to predict in a reliable way the pressure in the headspace and its trends during temperature changes.

c) Gas initially present in the headspace. The pressure of this gas increases with its temperature and with the reduction of the volume of the headspace. Direct proportionality to the number of moles and to the absolute temperature, and reverse proportionality to the actual volume may be predicted by many equations, including the perfect-gases-law.¹

As usual, scientific approaches call for a precise knowledge of the actual values of parameters and coefficients involved in the mathematical equations expressing the physical phenomena, otherwise the same equations are of no help for practical purposes. But the knowledge of such parameters and coefficients is in most cases unavailable. The following remarks do not contradict any element of the theoretical approach, but can provide a sound guide for decision-making in industrial practice.

¹ Scientific approaches to this problem may be found in Robert E. Beck, *Autoclaving of Solutions in Sealed Containers: Theoretical Pressure-Temperature Relationship*, *Pharmaceutical Manufacturing*/June 1985, pages 18 to 23, and in Martin A. Joyce & Jeffrey W. Lorenz, *Internal Pressure of Sealed Containers During Autoclaving*, *Journal of Parenteral Science and Technology*, Vol. 44 No. 6 / November - December 1990, pages 320 to 323.

Let us assume that:

- a) the liquid solution is an aqueous one; **different solutions may have a dramatically higher vapor pressure**;
- b) the aqueous solution is so diluted that its vapor pressure is that of the pure water (this assumption is a conservative one, as the more a solution is concentrated, the smaller becomes its vapor pressure, provided that no other changes occur);
- c) the total pressure in the head space of the sealed containers is 1.013 abs bar at the initial temperature;
- d) the gas filling the head space may be regarded as a perfect one, so that its pressure increases proportionally to the absolute temperature and in reverse proportionality to the volume of the head space;
- e) the head space is always at the thermodynamic equilibrium with the aqueous solution, i.e. their temperature is always the same;
- f) the initial temperature of the liquid inside the sealed container is 20 °C and the autoclaving temperature is 121 °C; this entails also that the initial pressure of 1.013 abs bar in the head space is the sum of 23 abs mbar of vapor pressure, depending only on the temperature, and of 0.990 abs bar of filling gas, depending on the mass amount, on the temperature and on the volume of the head space;
- g) the contribution of the escaping tendency of the dissolved gas may be forfeited to a value that, starting from naught, does not exceed 0.10 abs bar at 121 °C; this may be regarded as a conservative assumption for the case the dissolved gas is air (or pure nitrogen, or pure oxygen), **but cannot be assumed if the dissolved gas is carbon dioxide**; in this case, a detailed study should be necessary to predict the contribution of the carbon dioxide to the pressure in the head space: this would call also for the knowledge of the initially dissolved amount of gas;
- h) the container is rigid, and its volume is fix, but for the effect of the thermal expansions of its material;
- i) during the cooling inside the autoclave, the escaped gas do not re-dissolve in the liquid solution, at least until a temperature lower than 80 °C is reached; this conservative assumption corresponds with the current experiences, even if it slightly emphasizes them;

The thermal expansion of the volume of the pharmaceutical aqueous solutions is about 6 % between ambient temperature (specific volume of

pure water equal to about 1.00 l/kg) and 121 °C (specific volume of pure water equal to about 1.06 l/kg). The increase of internal volume of containers when temperature rises up depends remarkably on their material and on their shape; this increase is proportional to the third power of its linear expansion coefficient. There is no practical mean to prevent the thermal expansion of liquids during sterilization, as the pressure necessary for reducing of 6% the volume of liquid like water would be very large, i.e. of the order of magnitude of a thousand of bars, i.e. tens of thousands of psi.

As a rule of thumb, when containers of usual shape made by glass undergo a temperature change of about 100 °C, they increase their internal volume of about 1%, so that the reduction of the headspace inside a glass container filled with water roughly results in about the 5 % of the initial volume of the water. It is immediately evident that the pressure in the head space under autoclaving increases dramatically if the initial headspace is smaller. The table at the end of this article summarizes this fact.

A couple of final assumptions, slightly conservative as usual, intervene in the table:

- i) the reduction of head space at 100 °C and at 80 °C is considered to be the same as at 121 °C; this entails an over-evaluation of the total internal pressure of about 23 % in the worst case, that of 90 % filling level at 80 °C; both with lower filling levels and at higher temperature, the deviation becomes much smaller (less than 2 % with 50 % filling level at 80 °C);
- ii) the ambient pressure at sea level is 1.013 abs bar, and 0.813 abs bar at 800 m (2,600 ft) above the sea level.

A useful fact with glass containers is that between 20 °C and 121 °C, the mechanical resistance of the glass does not decrease appreciably.

Most of plastic materials known as polyethylene, and, for practical purposes, also most of those known as polypropylene, expand with the temperature increase to an extent that roughly compensate the thermal expansion of the water, so that the head space inside plastic containers might be assumed not to change when heated. However, the mechanical resistance of the plastics strongly decreases with the temperature, so that at 121 °C they can only withstand a differential pressure (i.e. a difference of pressure between their inside and their outside)

considerably smaller than the differential pressure they could withstand at ambient temperature.

The by far biggest component of the total pressure in the headspace of the sealed container at 121 °C is in all practical cases the vapor pressure of the liquid solution and this does not depend on the volume of the headspace, but only on the actual temperature of the solution. This happens thanks to the well-known one-to-one correspondence between pressure and temperature in the vapor and liquid equilibria.

The table at the end of the article contains some other assumptions and summarizes the result of basic calculation on internal pressure during autoclaving.

It is noteworthy that the differential pressure between the containers and the ambient is at 100° C very similar to the differential pressure during autoclaving at 121° C, but the mechanical stress on glass containers is much bigger while unloading. To the mechanical stress has to be added the so-called "thermal stress", due to the difference of temperature between the outer surface of the containers and the inner one. This combined stress may result in a sudden explosion of the total mass of the containers, even if during the autoclaving they have withstood the same differential pressure, or a bigger one. The effect of reducing the initial headspace is also dramatic, and the risk does much increase if the filling level overtakes 85%. The reduction of the ambient pressure at higher levels on the sea has to be considered as well.

If the unloading temperature is reduced, e.g. to 80 °C, the differential pressure decreases by 45 % to 70 %. This makes the unloading operation less dangerous, as both the risk of the explosion, the spray-around of the water, and the effect of the water on the human body would be reduced at a remarkable extent. However, with higher filling levels, only an unloading temperature of 65 °C or less should be regarded as reasonable safe, as the differential pressure should be smaller than 1 bar.

Filling levels above 90 % have not been considered for rigid containers, as they would quickly result in a very high pressure in the headspace, and this would make impossible the autoclaving.

On the contrary, very high filling levels do not hinder autoclaving of pre-filled syringes with a movable plug, since the internal volume of the syringes is in this case variable and may fully compensate for the thermal expansion of the solution. In any case, a suitable length of the syringe shall be provided to allow the plugs moving without any part of them ever protruding

outside the syringe itself, because this would involve a final and unacceptable entrainment of water in the outer groove(s) of the plugs.

| VLo | Initial cold conditions | | | | | Hot conditions | | | | |
|-------------------|-------------------------|---------|---------|---------|---------|----------------|-------------|-------------|-------------|-------------|
| | 50% | 70% | 80% | 85% | 90% | (50%) | (70%) | (80%) | (85%) | (90%) |
| HSo | 50% | 30% | 20% | 15% | 10% | (50%) | (30%) | (20%) | (15%) | (10%) |
| VL121 | (53.0%) | (74.2%) | (84.8%) | (90.1%) | (95.4%) | 53.0% | 74.2% | 84.8% | 90.1% | 95.4% |
| HS121 | (48.0%) | (26.8%) | (16.2%) | (10.9%) | (5.6%) | 48.0% | 26.8% | 16.2% | 10.9% | 5.6% |
| Δ HS121 | - | - | - | - | - | -4% | -11.1% | -19.0 | -27.3% | -44% |
| Δ Ppg | - | - | - | - | - | +4.2% | +11.9% | +23.5% | +37.6% | +78.6 |
| Pv121 | (0.023) | (0.023) | (0.023) | (0.023) | (0.023) | 2.05 | 2.05 | 2.05 | 2.05 | 2.05 |
| Pv100 | (0.023) | (0.023) | (0.023) | (0.023) | (0.023) | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |
| Pv80 | (0.023) | (0.023) | (0.023) | (0.023) | (0.023) | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 |
| PEG | (0.0) | (0.0) | (0.0) | (0.0) | (0.0) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Po121 | (0.990) | (0.990) | (0.990) | (0.990) | (0.990) | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 |
| Ppg121 | - | - | - | - | - | 1.39 | 1.49 | 1.64 | 1.83 | 2.36 |
| Ptot121 | - | - | - | - | - | 3.54 | 3.64 | 3.79 | 3.98 | 4.51 |
| Δ P121 | - | - | - | - | - | 1.49 | 1.59 | 1.74 | 1.93 | 2.46 |
| Po100 | (0.990) | (0.990) | (0.990) | (0.990) | (0.990) | 1.26 | 1.26 | 1.26 | 1.26 | 1.26 |
| Ppg100 | - | - | - | - | - | 1.31 | 1.41 | 1.56 | 1.73 | 2.25 |
| Ptot100 | - | - | - | - | - | 2.42 | 1.42 | 2.67 | 2.84 | 3.36 |
| Δ P100SL | - | - | - | - | - | 1.41 | 1.51 | 1.66 | 1.83 | 2.35 |
| Δ P100/800 | - | - | - | - | - | 1.49 | 1.59 | 1.74 | 1.91 | 2.43 |
| Po80 | (0.990) | (0.990) | (0.990) | (0.990) | (0.990) | 1.19 | 1.19 | 1.19 | 1.19 | 1.19 |
| Ppg80 | - | - | - | - | - | 1.24 | 1.33 | 1.47 | 1.64 | 2.13 |
| Ptot80 | - | - | - | - | - | 1.81 | 1.90 | 2.04 | 2.21 | 2.70 |
| Δ P80SL | - | - | - | - | - | 0.80 | 0.89 | 1.03 | 1.20 | 1.69 |
| Δ P80/800 | - | - | - | - | - | 0.88 | 0.97 | 1.11 | 1.28 | 1.77 |

Legend

| | |
|------------------|---|
| VLo | Filling level in the cold container |
| Hso | Head space in the cold container |
| VL121 | Filling level in the hot container, referred to the cold container |
| HS121 | Head space in the hot container, referred to the cold container |
| Δ HS121 | Change of the head space volume from cold to hot container |
| Δ Ppg | Change of perfect gas pressure inside the head space from cold to hot conditions |
| (0.023) | Vapor pressure of pure water at 20°C, abs bar |
| (0.990) | Pressure of the initial amount gas in the initial head space, that fills the head space to the total pressure of 1.013 abs bar at 20°C, abs bar |
| (0.0) | Pressure of the gas escaped from the liquid into the head space at 20°C, abs bar |
| Pv121 | Vapor pressure of pure water at 121°C, abs bar |
| Pv100 | Vapor pressure of pure water at 100°C, abs bar |
| Pv80 | Vapor pressure of pure water at 80°C, abs bar |
| PEG | Pressure of the gas escaped under hot conditions into the head space, abs bar |
| Po121 | Pressure of the initial amount of gas in the initial head space at 121°C, abs bar |
| Ppg121 | Pressure of the initial amount of gas in the reduced head space at 121°C, abs bar |
| Ptot121 | Total pressure in the reduced head space at 121°C, abs bar |
| Δ P121 | Differential pressure between sealed container and autoclave at 121°C, bar |
| Po100 | Pressure at 100°C of the initial amount of gas in the initial head space, abs bar |
| Ppg100 | Pressure at 100°C of the initial amount of gas in the reduced head space, abs bar |
| Ptot100 | Total pressure in the reduced head space at 100°C, abs bar |
| Δ P100/SL | Differential pressure between sealed container at 100°C and ambient at sea level (ambient pressure = 1.013 abs bar), bar |

$\Delta P_{100/800}$

Differential pressure between sealed container at 100°C and ambient, at 800 m above the sea level (ambient pressure: 0.813 abs bar), bar

P_{o80}

Pressure at 80°C of the initial amount of gas in the initial head space, abs bar

P_{pg80}

Pressure at 80°C of the initial amount of gas in the reduced head space, abs bar

P_{tot80}

Total pressure in the reduced head space at 80°C, abs bar

ΔP_{80}

Differential pressure between sealed container and ambient at sea level (ambient pressure = 1.013 abs bar), bar

$\Delta P_{80/800}$

Differential pressure between sealed container at 80°C and ambient, at 800 m (2,600 ft) above the sea level (ambient pressure = 0.813 abs bar), bar