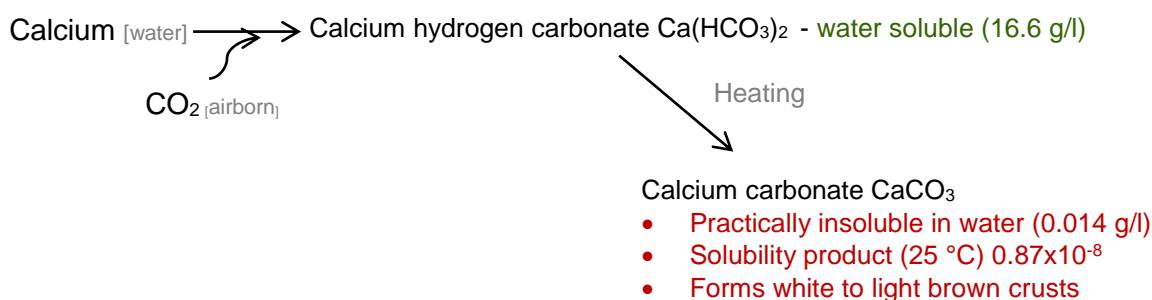


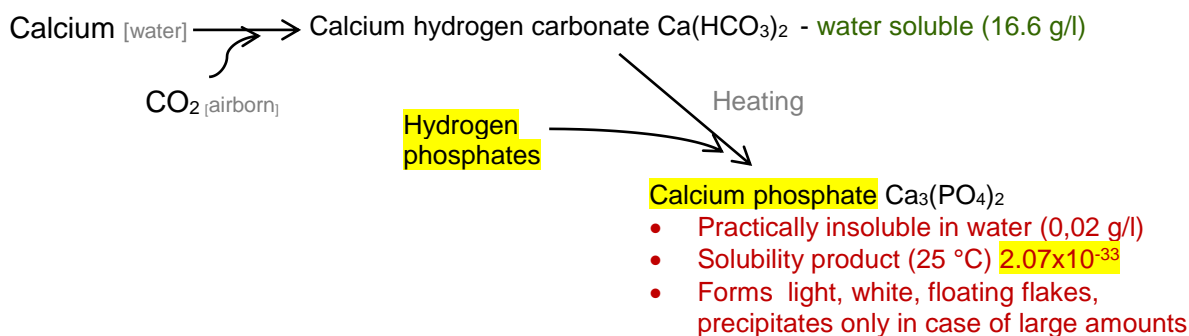
# Clouding of Microbiological Media

In few rare cases, clouding of microbiological broths happens naturally, resulting from certain ingredients which do not solubilise completely during preparation (for instance cetrimide).  
 In most cases, clouding is the result of small residues of calcium, which reacts with added phosphates, forming insoluble salts.

## 1.) Precipitation of Chalk w/o presence of Phosphates



## 2.) Formation of Insoluble Calcium Phosphates under Presence of Phosphate

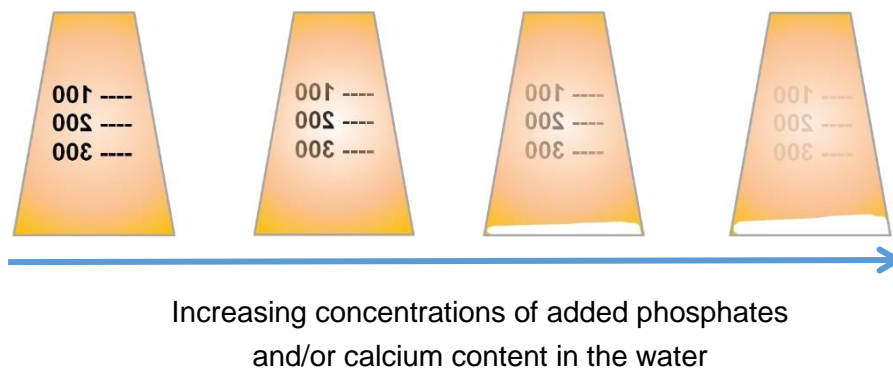


### Example: Terrific-Broth

Casein, enzymatically digested	.....	12 g/l
Yeast extract	.....	24 g/l
$\text{K}_2\text{HPO}_4$	.....	12.5 g/l
$\text{KH}_2\text{PO}_4$	.....	2.3 g/l

## Technical Info

In small amounts, calcium phosphates are distributed in the broth, only appearing as clouding of the – normally clear – broth. Assayed from the back, the labelling of those bottles or Erlenmeyer flasks appears diffuse and not distinct. Only when they reach a certain critical amount, the phosphates form a visible precipitate, or appear as white clouds.



### Typically Found in the Following Media (Boths with High Phosphate Content)

TB, Peptone Water, Fraser Half Broth, Fraser Broth, Mossel Enrichment Broth

### Rarely Found in the Following Media (Broths with low Phosphate Content)

CASO Bouillon, Coli Fluoro Broth, Brain Heart Infusion Broth, Laurylsulphate Broth, MRS Broth

### Please Note

With extracts (particularly yeast or meat extract) phosphates are also added to the broth.

Due to batch variability of those natural products, some batches may form more severe clouding than others.

### In Order to Prevent this Effect We Recommend the Following Procedure

- A) In the vast majority of cases, phosphate reduction of the medium composition due to precipitation of phosphates doesn't reduce the quality of the broth or bacterial growth rates. In case the clouding doesn't hinder the downstream processes (OD measurement or such), the broth may be used just as it is.
- B) In case the precipitates shall be removed, we recommend filtration through filter membranes with large pores (several  $\mu\text{m}$ ). Please make sure, filtration units and broth are handled sterile.
- C) Filtration prior to autoclaving is not sufficient, since due to the heating during steam sterilisation phosphate salts form anew.
- D) Try to reduce phosphate concentration of the broth. Instead of Terrific Broth, one can use the phosphate reduced version *Terrific Broth, modified* (Art. No. HP61).
- E) Check your water desalting or distillation unit. Analyse the calcium content (water hardness) of your water (see Roth water test sticks).



Well advised with Roth.

## Technical Info

### Solubility Products of Some Phosphates and Other Salts

Salt	Formula	Temperature	Solubility Product (Mol <sup>2</sup> /Litre <sup>2</sup> )
Aluminium hydroxyde (anhydrous)	Al(OH) <sub>3</sub>	20 °C	1,9 x 10 <sup>-33</sup>
Cadmium phosphate	Cd <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	25 °C	2,53 x 10 <sup>-33</sup>
Calcium carbonate	CaCO <sub>3</sub>	25 °C	0,87 x 10 <sup>-8</sup>
Calcium phosphate	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	25 °C	2,07 x 10 <sup>-33</sup>
Calcium sulfate	CaSO <sub>4</sub>	25 °C	4,93 x 10 <sup>-5</sup>
Cobalt phosphate	Co <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	25 °C	2,05 x 10 <sup>-35</sup>
Iron (II) phosphate	Fe <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> x 8 H <sub>2</sub> O	25 °C	1,70 x 10 <sup>-29</sup>
Potassium chloride	KCl	20 °C	21,25
Potassium perchlorate	KClO <sub>4</sub>	25 °C	1,05 x 10 <sup>-2</sup>
Copper (II) phosphate	Cu <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	25 °C	1,4 x 10 <sup>-37</sup>
Copper (II) sulphide	CuS	25 °C	8 x 10 <sup>-37</sup>
Lithium carbonate	LiCO <sub>3</sub>	25 °C	1,7 x 10 <sup>-3</sup>
Lithium fluoride	LiF	25 °C	1,84 x 10 <sup>-3</sup>
Lithium phosphate	Li <sub>3</sub> PO <sub>4</sub>	25 °C	2,37 x 10 <sup>-4</sup>
Mercury (II) sulphide	HgS	25 °C	2 x 10 <sup>-53</sup>

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s.s. 02/2016