

# Affinity Membranes: A Tool to Remove Pathogens

Affinity membranes can selectively remove bacteria, endotoxins and viruses from biologically active liquids and water. Their use is set to rise. The mechanism and capabilities of the technology are described.

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## Improved removal efficiency

Increasing concerns about cleaner and safer filtration in general, and in the healthcare context, concerns about health, nutrition and economics are expected to consolidate the demand for filtration. The need will grow for innovative filters made using cutting-edge technology that can maintain the low cost of filters and their production. This article outlines the concepts on which affinity membranes are based and the capabilities of the related filters. Specifically, biomimetically functionalised membranes are described. These cellular membranes are able to selectively capture pathogens (viruses, endotoxins and bacteria) from biologically active liquids and water. The performance of these new membranes is compared with the performance of traditional sterilisation/purification technologies.

The need to remove known or unknown pathogens from biologically active liquids and water is a major challenge in order to comply with regulatory requirements and guarantee microbiological safety. Pathogen particles include bacteria that range in size from 0.3 to 10  $\mu\text{m}$ , viruses that range in size from 0.02 to 0.2  $\mu\text{m}$ , and endotoxins that typically range from 0.005 to 0.02  $\mu\text{m}$ .

Convective sieving and adsorption control removal efficiency and flow rate through affinity membranes. Compared with traditional ultrafiltration membranes that operate by a size exclusion mechanism, affinity membranes are characterised by higher specificity and higher flow rate.



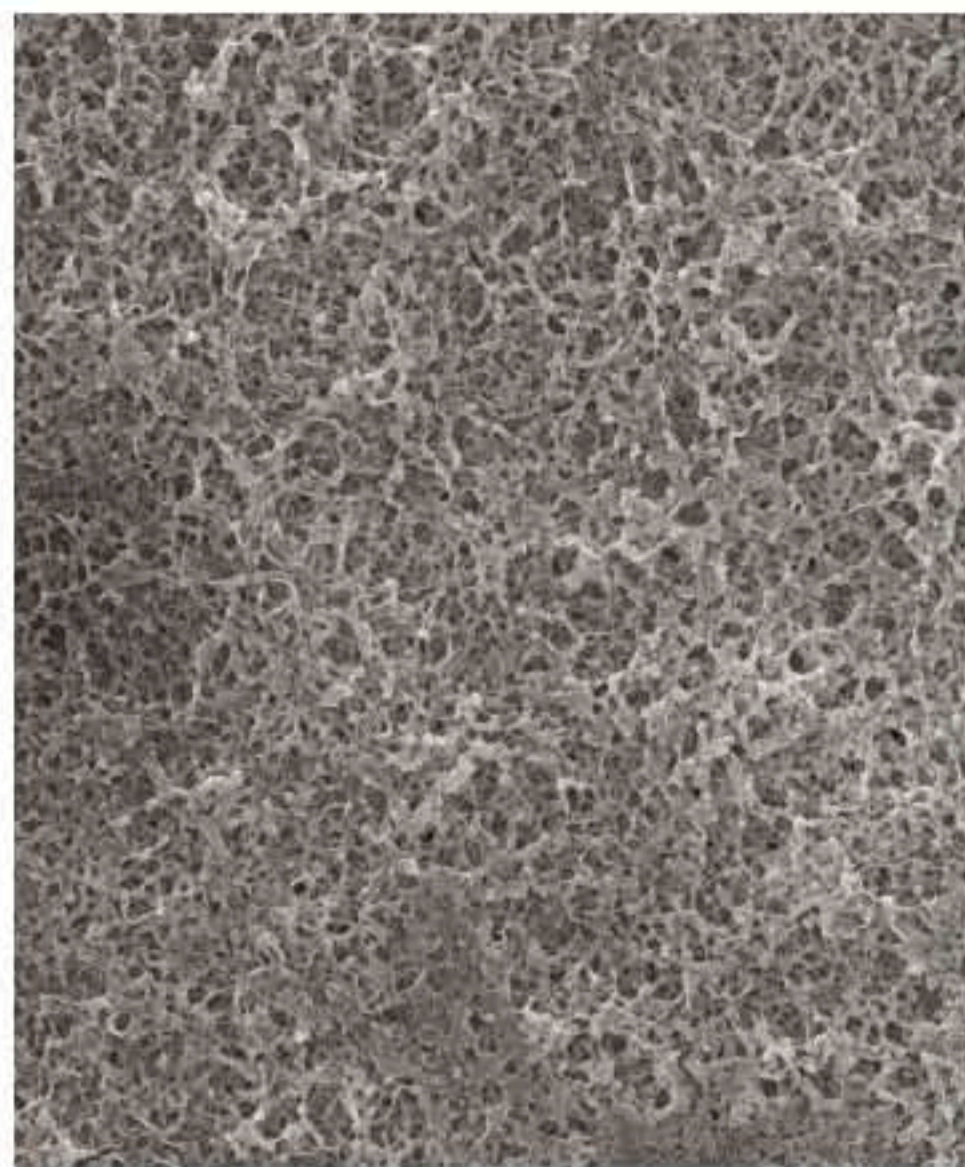
Compared with affinity resins, these membranes operate more homogeneously and at flow rates approximately 200 times faster than resins.<sup>1</sup> Therefore, affinity membranes combine the high selectivity of resins and the high flow rate of microporous membranes. This is an important achievement that changes the traditional paradigm that states as flow rate increases, membrane efficiency decreases.

## The affinity filtration mechanism

Affinity membranes are composed of functional groups (ligands) that are attached to the surface of the pores of a microfiltration membrane. The specific surface area of a microfiltration membrane normally

used for conventional filtration provides a maximum amount of binding sites where tailored functional groups are attached.<sup>2</sup> Figure 1 is a scanning electron microscope (SEM) image of the cross-section of a microfiltration membrane. It shows the large area of the wall surface of the interconnected pores that is available to be functionalised. Examples of functionalising groups that are able to guarantee high selectivity towards viruses and endotoxins are amino acids, peptides, glycoproteins, glycosaminoglycans, synthetic glycosylated copolymers, quaternary ammonium salts, amines, organic polymers and inorganic materials. When the microfiltration membrane is functionalised with these groups,

**FIGURE 1:** SEM image of a microfiltration membrane.



the functionalised sites are easily accessible by small pathogens such as most viruses and endotoxins. During filtration, viruses and endotoxins are captured when the feed solution flows through the membrane; sieving separates bacteria, which are bigger than the membrane pore size of typically 0.2 µm.

Figure 2 schematically illustrates the mechanism. In this example, the ligands are positively charged active groups that are bonded to the surface of the pores of the membrane; the small spheres represent the negatively charged endotoxins or viruses that have dimensions smaller than the pore diameter of the membrane. Normally, they would pass through the bigger membrane pores, but because of the presence of electrostatically active ligands they are captured and do not pass through the membrane. Bacteria, which are a size bigger than the nominal membrane pore size, do not pass through. The same result can be obtained by functionalising the surface of the membrane pores with alternative molecules characterised by a high affinity to viruses and endotoxins.

By optimising the ligand type, stability and density, it is possible to achieve high separation efficiency. Simultaneously, the pressure drop is kept low by optimising the membrane pore size and this can guarantee high flow rate and sufficient pathogen

adsorption capacity, which is proportional to the functionalised surface area of the pores.

### Multifunctional filters

Affinity membranes have proven to be effective materials for multifunctional filters that are characterised by high bacteria, endotoxins and viruses retention; high flow rate; and improved capacity and flow uniformity. The filters include different sections, each made up of specifically functionalised multiple layers. For example, there could be a polycationic-type section devoted to the removal of negatively charged pathogens, followed by a polyanionic section devoted to the removal of positively charged pathogens. Their efficiency is usually measured by the log removal value (LRV):  $LVR = \log_{10}(C_{in}/C_{out})$ , where C is the pathogen concentration.

There are a number of filters of this type on the market. For example, there are membrane adsorbers for rapid purification of proteins (Sartorius AG, [www.sartorius.com](http://www.sartorius.com)); capsules and cartridges consisting of microporous membranes onto which is grafted a synthetic polymer containing sulphonic or carboxylic acid groups (Pall Corp, [www.pall.com](http://www.pall.com)); and a filter that uses a positively charged 0.2 µm polyethersulphone membrane to remove bacteria and endotoxins from injectable liquids (GVS SpA, [www.gvs.com](http://www.gvs.com)). All these filters typically operate at pressures between 0.05 and 0.5 bar and are characterised by

- virus removal efficiency equal to a LRV of >5 for viruses bigger than 50 nm and an LRV of >4 for viruses bigger than 20 nm
- an endotoxin removal efficiency higher than 80% after 96 h of operation
- resistance to gamma irradiation, ethylene oxide and steam sterilisation
- biocompatibility according to ISO 10993, Biological Evaluation of Medical Devices.

### Affinity versus traditional methods

The advantages that these filters offer are even clearer when the limitations and the drawbacks of traditional treatments are considered. Traditionally, pathogens are →



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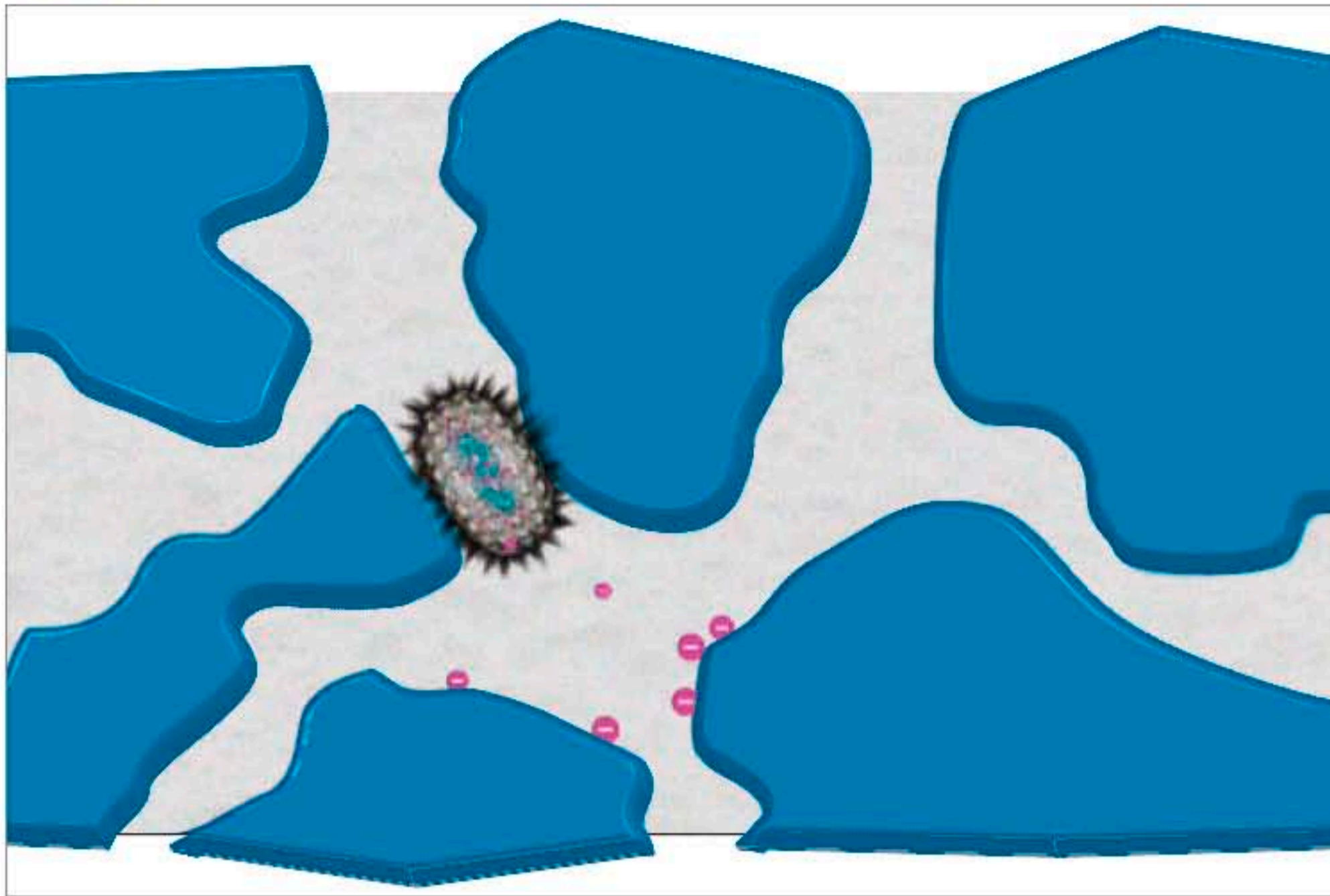


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FIGURE 2: Schematic diagram of an affinity membrane.



→ treated by inactivation or removal. The inactivation methods include ultraviolet (UV) disinfection, heating and chemical disinfection. The removal methods include membrane filtration and chromatography. The UV disinfection disrupts the biological cells and is suitable for all pathogens, except the most resilient viruses.

Heating effectively inactivates viruses and whole bacterial cells. However, it eliminates bacterial endotoxins only after heating for long periods at elevated temperatures (250 °C for 30 min); this method cannot be applied to heat-sensitive biological materials.

Solvent treatment is a commonly used method for viral inactivation of biologicals. The treatment disrupts the lipid envelope and destroys the ability of the virus to bind to cells and replicate. Therefore, the solvent method cannot be used on viruses without a lipid envelope. Moreover, when using inactivation methods, the pathogens and chemical solvents that are used remain in the final product and need to be removed.

Reverse osmosis (RO), ultrafiltration (UF), nanofiltration (NF), and microfiltration (MF) membranes are used routinely to eliminate pathogens. MF membranes with nominal pore size equal to 0.2 µm effectively remove bacteria that are bigger

in size than 0.2 µm, but generally, removal of viruses and endotoxins is poor (they are smaller than 0.2 µm), because the removal mechanism is sieving by physical exclusion. UF, NF and RO effectively remove viruses

Affinity membrane filters **operate at pressure lower** than the pressure required by ultrafiltration, nanofiltration and reverse osmosis processes.

and endotoxins. However, the operating pressure of UF (2–8 bar), NF (7–15 bar) and RO (10–80 bar) is much higher than the operating pressure of MF affinity membranes (0.05–0.5 bar); this involves the use of pumps to obtain the same flow rate of affinity membranes. Moreover, because of occasional membrane defects, the viral removal efficiency is not always guaranteed.

Chromatography is generally limited by low capacity and long purification times

because of the slow diffusion of the liquid when it enters and exits from the pores of the resin. Usually, this method is not as efficient because resins in the chromatographic columns become packed as a result of channelling and by-pass phenomena, which leads to different absorption and desorption rates, high cost and higher pressure drop in the packed beds (the liquid to be purified encounters higher resistance to access the active sites of the resins and the purification process requires a long time to purify the liquid).

In contrast, affinity membrane filters are capable of capturing viruses and endotoxins at extremely higher flow rates with the efficiency typical of chromatography resins. They eliminate the slow diffusion times typical of chromatography, speeding up the purification processes, and they operate at pressure lower (less than 0.1 bar) than the pressure required by UF, NF and RO processes. They seem to offer significant advantages in cost and time reduction over traditional pathogen treatment methods.

Up to now they are used to treat injectable liquids and their use in pharmaceutical and biotechnology downstream applications has been limited. However, they promise important future applications for the sterilisation of challenging liquids by combining the best characteristics of chromatography and traditional membrane filtration. ☺

### References

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