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# Loading and controlled release of antibiotics from biomaterials for bone regeneration

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**Summary:** Commercially available antibiotics can be mixed with the biomimetic bone regeneration materials from the **CERASORB®** product family. The assays show that, due to both the loading capacity of the materials as well as the release kinetics, the concentrations of active substances over time are always above the MIC (minimum inhibitory concentration) and below the toxic threshold in the environment of the biomaterials. Release tests in media similar to the human body, e.g. fetal calf serum (FCS), suggest that the release is much slower in such media, meaning the active substance is available for a longer period of time.

*Keywords: Cerasorb®, antibiotics, release kinetics, infection prophylaxis*

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## 1. Introduction

Perioperative antibiotics are administered in routine surgical procedures as a prophylaxis to prevent infection or the formation of a biofilm. This prophylaxis must be loaded early enough and in sufficiently high doses to ensure there is an adequate concentration of antibiotics at the site where the operation is to be performed.

The intraoperative local use of antibiotics has become common practice in orthopedic and trauma surgery since the introduction of antibiotic- [gentamicin-] loaded PMMA cements and chains, in particular for treating infections that have already been confirmed.

However, a major disadvantage of these substances is that the material then needs to be removed after the antibiotics have been released. Another major drawback is that, right from the start, the practitioner is limited to using a specific antibiotic at a certain dosage and is unable to adapt it to the specifics of the respective patient and case.

The aim, therefore, is to be able to freely combine resorbable materials for bone regeneration with an antibiotic in order to facilitate one of the major objectives, the so-called „burst release“, i.e. the highest possible release of the antibiotic within 72 hours. This should result in the continuous and verifiable release of the antibiotic over a minimum period of 48 hours.

There is a wide range of bone replacement materials of different origins and compositions and with different absorption kinetics.

**CERASORB® M** granules made from beta-tricalcium phosphate (β-TCP) are available in different granule sizes and are used throughout the entire skeletal system. The interconnecting pore system allows for progressive angiogenesis and vascularization, thereby ensuring active cellular infiltration throughout the entire resorption process.

**CERASORB® Foam** is a highly porous composite made of porcine collagen (type I) and pure-phase β-TCP granules of different sizes and densities. It has a granular fraction of 85 % by weight, which gives it high volume stability after breakdown of the faster resorbing collagen. The material is completely absorbed over a matter of months and replaced by the body‘s own bone.

The results of the in vitro loading tests and the release kinetics of various antibiotics from **CERASORB® M** granules and **CERASORB® Foam** are presented below. An important aspect is the minimum inhibitory concentration or MIC, i.e. the minimum concentration of the active substance which must be present in order to achieve the desired antibacterial activity.

## 2. Materials and Methods

curasan AG provided granular biomaterials made from β-TCP **(CERASORB® M)** in the granule sizes 1000 – 8000 µm for bone regeneration, as well as composite materials made from porcine collagen (type I) and β-TCP (**CERASORB® Moldable Foam**, malleable, density 0.1 – 0.3 g/cm³ and **CERASORB® Flexible Foam Strip**, permanently elastic, density 0.3 – 0.5 g/cm³). The antibiotic solutions were prepared according to the antibiotic manufacturer's specifications as described in table 1.

| Description | LOT | Quantity | Quantity of water* | Yield mg/mL | MHK/MIC [µg/mL] |
|---|---|---|---|---|---|
| Vancomycin HIKMA CP 1 g | AK0127 | 1 g | 20 mL | 50 | 2 |
| Meropenem HIKMA 1 g | MDEA 1068 | 1 g | 20 mL | 50 | 2 |
| Gentamicin Ratiopharm 80 mg/2 mL SF finished solution in ampoules | T18942A | 80 mg | 2 mL | 40 | 1 |

**Table 1:** Antibiotics used. * Water for injections

### 2.1 Loading tests

1cc of biomaterial was introduced to each solution, and the solutions were then added in 100 µL increments using a precision pipette until the liquid was no longer absorbed. The loading capacity was then calculated as a percentage and compared with commercially available materials.

### 2.2 Release tests

The samples were loaded to saturation according to the capacity determined. After a 5-minute reaction time, the samples were incubated at 37 °C in beakers containing 20 mL of 0.9 % saline solution. The release solution was first replaced every 30 minutes, and these intervals were then extended. The quantitative photometric measurement of the release was carried out using a Tecan Microplate Reader.

## 3. Results

### 3.1 Loading tests

Figure 1 shows the percentage loading capacity compared with calcium sulfate (gypsum) products for the release of antibiotics. In particular, the composite materials can absorb their own volume of liquid many times over, and can therefore be loaded with the active substance in such a way that it corresponds to the MIC.

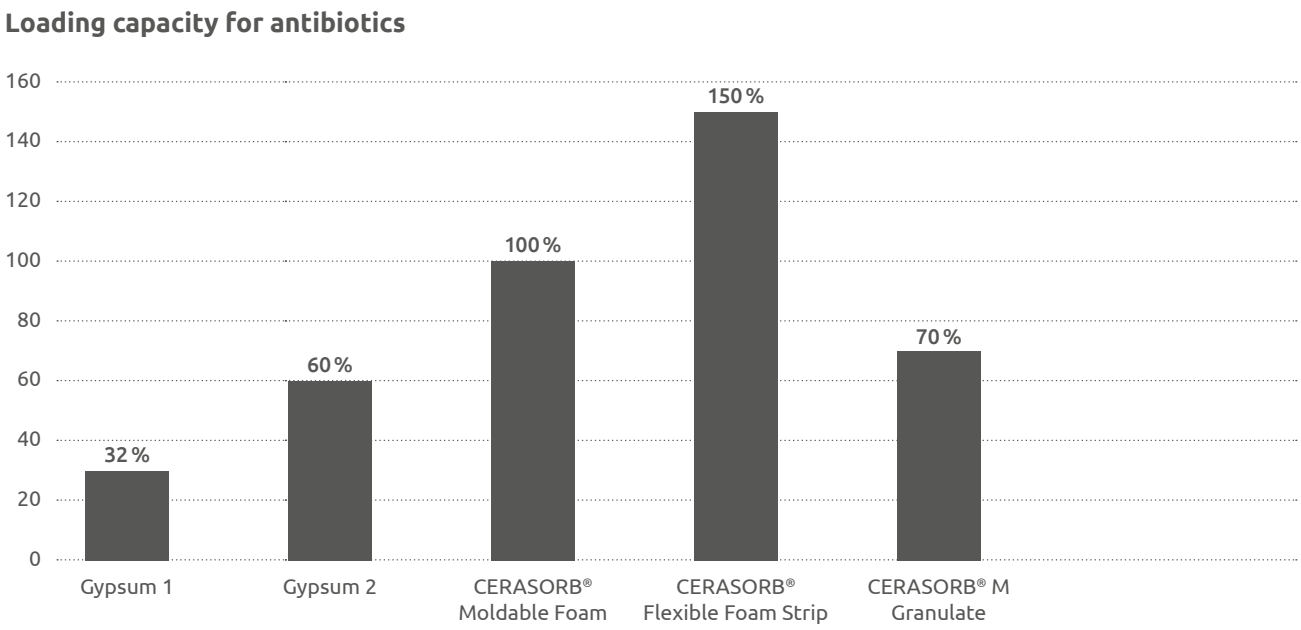


**Figure 1:** Loading capacity of the biomaterials as a percentage compared with calcium sulfate products (values for the gypsum products were obtained from the promotional materials of the respective manufacturers)

The powdered antibiotics Meropenem and Vancomycin can be used in correspondingly higher concentrations to increase the loading of the substrate (Table 2).

| | VANCOMYCIN | | MEROPENEM | | GENTAMICIN | |
|---|---|---|---|---|---|---|
| Description | mL per cc matrix | mg per cc matrix | mL per cc matrix | mg per cc matrix | mL per cc matrix | mg per cc matrix |
| **CERASORB®** Flexible Foam | 1.5 | 75.0 | 1.5 | 75 | 1.5 | 60.0 |
| **CERASORB®** Moldable Foam | 1 | 50.0 | 1 | 50 | 1 | 40.0 |
| **CERASORB®** Granulate 1000–2000 µm | 0.65 | 32.5 | 0.63 | 31.6 | 0.65 | 26.0 |
| **CERASORB®** Granulate 2000–3000 µm | 0.51 | 25.3 | 0.52 | 26.2 | 0.54 | 21.7 |
| **CERASORB®** Granulate 3000–5000 µm | 0.31 | 15.4 | 0.34 | 17.2 | 0.38 | 15.3 |
| **CERASORB®** Granulate 5000–8000 µm | 0.62 | 31.2 | 0.61 | 30.3 | 0.62 | 24.9 |

**Table 2:** Loading of the biomaterials with active ingredient solution in mL liquid per cc matrix and the resulting quantity of active ingredient in mg

### 3.2 Release tests

Figure 2 a-b shows the release of Gentamicin from **CERASORB® Foam** and **CERASORB® M** granules in the laboratory. 60 % of the Gentamicin was released from the Moldable Foam after 1.5 hrs, and only after 6 hrs from the Flexible Foam Strip.

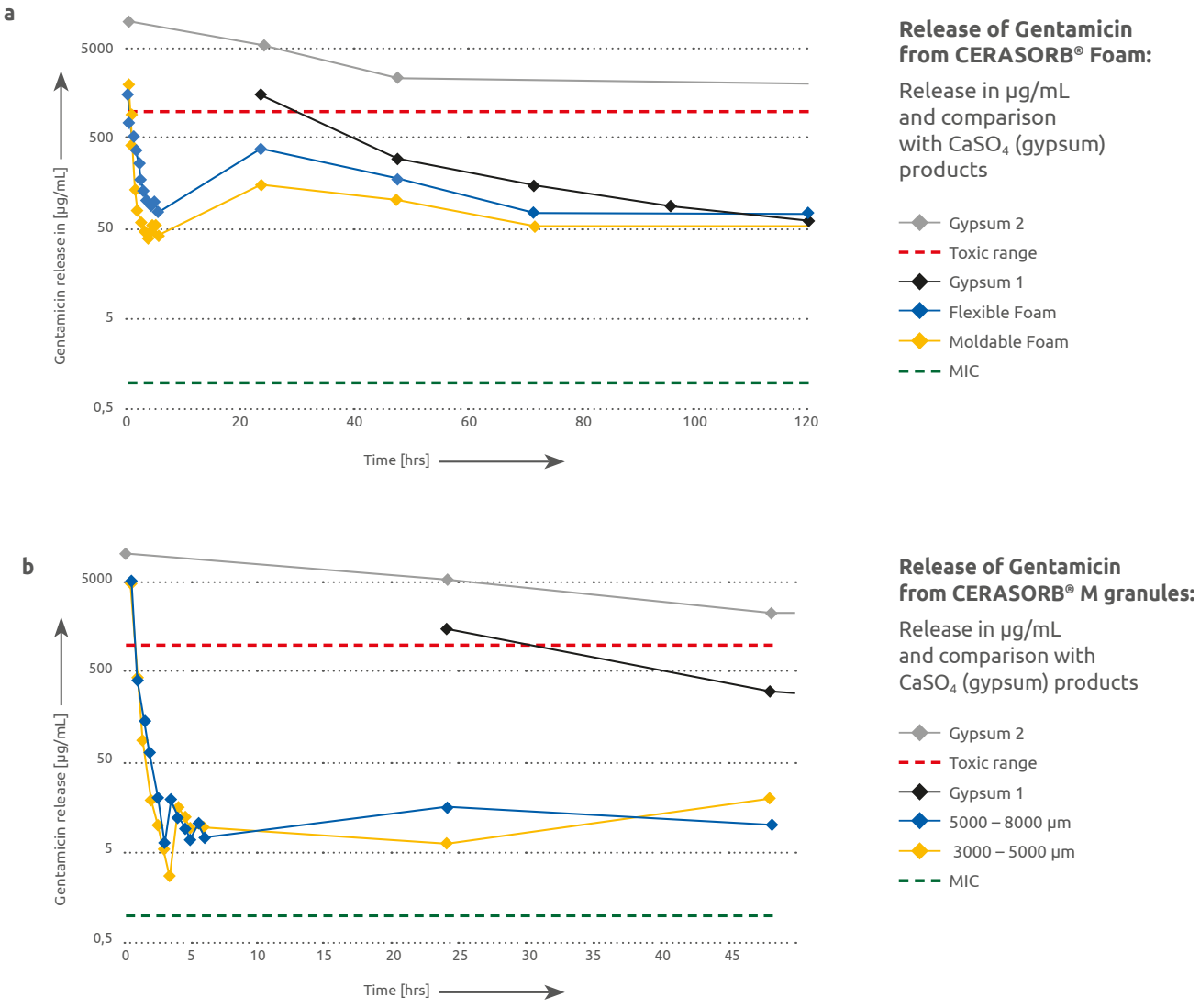


**Figure 2:** Release kinetics of gentamicin from **CERASORB® Foam** and **CERASORB® M** granules with minimal inhibitory concentration and toxic range.

After 120 hours approx. 80 % was released from the **CERASORB® Moldable Foam** and approx. 70 % from the **CERASORB® Flexible Foam Strip**. This means that after 5 days, 20 or 30 % of the active substance remains and is still available in the substrate. The values never fall below the MIC. The "initial burst" stage is completed after about an hour, meaning the concentration then remains below the toxic range.

Figure 2b shows that even after 48 hours, approx. 30 % of the antibiotic used is still bound in the 5000–8000 µm granulate, and approx. 10 % of the antibiotic in the 3000 – 5000 µm granulate. This is as a direct result of the structure of the granules: the porous structure of **CERASORB® M** is engineered with interconnected pores, allowing for a microporous system with a high surface area and capillary action. This increases the adhesion of the antibiotic solution to the granules. This residual amount of antibiotics then releases over a period of weeks during the absorption/degradation of the bone-building material.

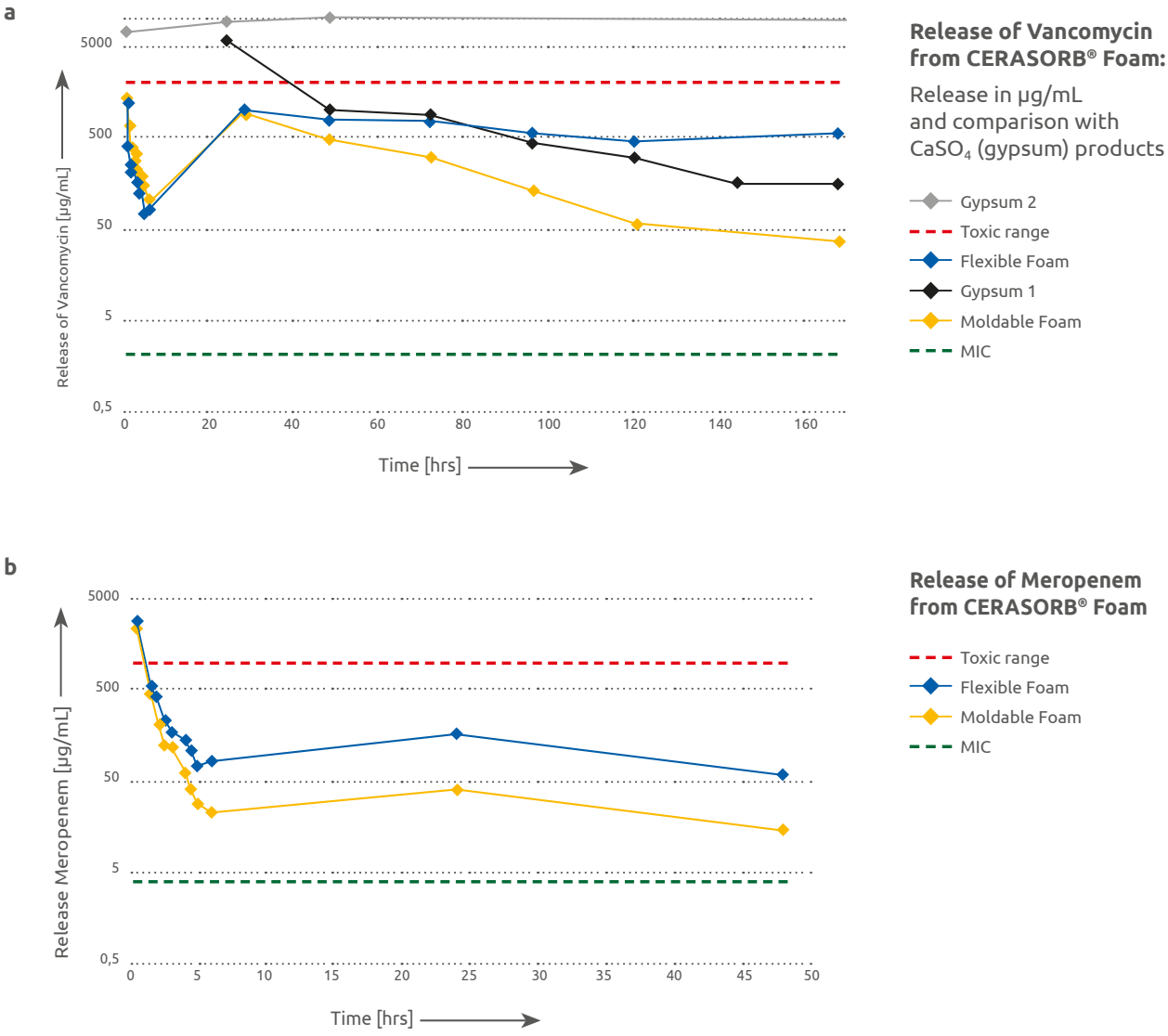


**Figure 3:** Release kinetics of a: Vancomycin and b: Meropenem from **CERASORB® Foam**.

Figure 3 shows the active ingredients Meropenem and Vancomycin. Again, apart from an initial higher concentration, the active ingredients are released over many days. It can be assumed that the active substances are adsorbed on the biomaterial in such a way that the active ingredients are released during the biodegradation and conversion of the material into the body's own bone.

## 4. Conclusion and outlook

Commercially available antibiotic preparations can be mixed with the biomimetic bone regeneration materials from the **CERASORB®** product family. The assays show that, due to both the loading capacity of the materials as well as the release kinetics, the concentrations of active substances over time are always above the MIC (minimum inhibitory concentration) and below the toxic threshold in the environment of the biomaterials. Release tests in media similar to the human body, for example in fetal calf serum (FCS), suggest that the release is much slower in such media, meaning the active substance is available for a longer period of time.

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