The importance of the formulation in a CHX + CPC mouthrinse like Perio-Aid.

The addition of Chlorhexidine and its salts to existing treatments is, without a doubt, a very important milestone. Specifically, the oral health world has benefited greatly by achieving complete control and prevention of diseases caused by pathogenic microorganisms, such as gingivitis and its more severe outcome, periodontitis.

Today Chlorhexidine is still considered to be the "Gold Standard" for treatment of these diseases.

As commonly occurs, the discovery of Chlorhexidine's potential as an antiseptic was a coincidence stemming from research conducted by the British Laboratory, Imperial Chemical Industries (ICI), on the biological properties of polyguanidines to find active molecules to fight malaria.

The discovery of this compound's great antiseptic activity was disclosed to the scientific community way back in 1954 in an article published in the Brit.J. Pharmacol ([1954],9,192), whose original title information is displayed below:

Brit. J. Pharmacol. (1954), 9, 192.

1:6 –DI-4'- CHLOROPHENYLDIGUANIDOHEXANE ("HIBITANE"*). LABORATORY INVESTIGATION OF A NEW ANTIBACTERIAL AGENT OF HIGHT POTENCY

BY

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(RECEIVED JANUARY 7, 1954)

Fig. 1

This study not only helped to confirm the elevated antiseptic activity against different bacteria, but also strongly validated a set of facts regarding the activity-structure relationship of this class of compounds, therefore permanently determining the structural details of the current Chlorhexidine which in the ICI study was given derivative code number 10.040.

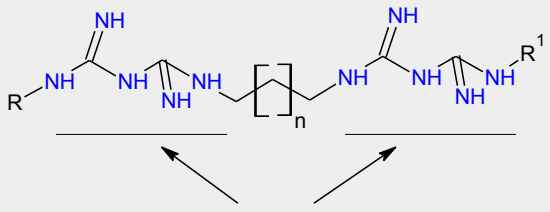
The Table below, taken from the article, definitively clarifies which of the numerous synthesised compounds had the best antiseptic activity, which was measured using 3 different bacterial strains:

TABLE I
COMPARATIVE BACTERIOSTATIC ACTIVITY OF A NUMBER OF AGENTS RELATED TO 10,040
Compounds of type: R.NH.C.NH.C.NH(X)NH.C.NH.C.NH.R
NH NH NH NH

Code No.	Terminal Group R	Central Unit X	Comparative Bacteriostatic Effect		
			Bact. Coli	*Staph. Aureus*	*Ps. Pyocyanea*
12,483	4-Chlorophenyl	Trimethylene	0·3	1	0·3
10,040	"	Hexamethylene	1	1	1
11,383	"	Decamethylene	0·3	0·3-1	<0·01
11,385	"	(4:4′)-Diphenylmethane	1	0·3	0·3
11,384	"	(1:4)-Phenylene	0·1	0·1	0·03
10,387	Phenyl	Hexamethylene	0·3-1	0·3-1	0·1
11,386	3:4-Dichlorophenyl	"	0·3	0·3	1
11,108	4-Hydroxiphenyl	"	0·03	0·01	<0·01
10,689	4-Methoxiphenyl	"	0·3	0·1	0·01
10,691	4-Carboxiphenyl	"	<0·01	<0·01	<0·01
9,381	RNH.=Et2N	"	0·1	0·3	<0·01
14,575	RNH.=4-ClCXHXNMe-	"	0·3	1	0·1
10,030	RNH.C.=hydrogen NH	"	<0·01	<0·01	<0·01
11,717	RNH.C.=4-chlorophenyl NH	"	0·03	0·03	0·03

Fig. 2

The general structure of derivative code 10.040 was as follows:



DIGUANIDINE GROUPS
If R = R1 = p-chlorophenyl and n = 4, the molecule is CHLORHEXIDINE

Fig. 3

The optimum distance between the diguanidine groups in terms of units -CH_2- (or methylene groups) was found to be 6 units, and the best R replacement group at each end of the molecule was a p-chlorophenyl.

And now, after much discussion, we have a "photograph" of our beloved and much appreciated antiseptic in the form of di-gluconate salt, one of the most soluble of those commercially available:

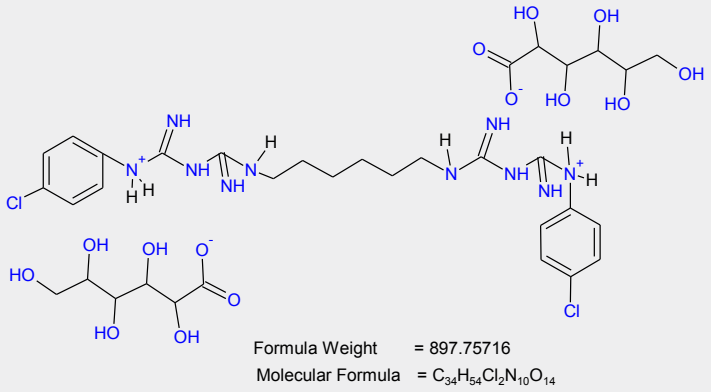


Fig. 4

It is a truly impressive and complex molecule having high molecular weight with two positive charges on the nitrogen atom closest to the p-ChloroPhenyl due to salt formation with gluconic acid.

Chlorhexidine di-gluconate is the most commercially available salt for this active ingredient and is sold as a 20% aqueous solution.
From here onward, we will refer to Chlorhexidine di-gluconate as CHX.
After its synthesis and then its discovery as a powerful antiseptic, its trajectory over the years has been:

- **1950s** – Synthesis and discovery as a powerful antiseptic
- **1954** – CHX is marketed in the UK as a topical disinfectant and antiseptic.
- **1970s** – Washing hands with CHX proves to reduce bacteria by 90%. CHX is marketed in the USA.
- **1976** – CHX is proven to inhibit dental biofilm formation.
- **1980s** – Formulation of mouthrinses using 0.20% CHX at first and then 0.12% CHX.
- **1992** – Perioaid with 0.12% CHX is marketed .
- **From 1995** – Perioaid Treatment (0.12% CHX + 0.05% CPC) and Perioaid Maintenance (0.05% CHX + 0.05% CPC) are marketed.

Starting in the mid-seventies, CHX, a very powerful antiseptic and anti-plaque agent is available as an active ingredient, capable of preventing gingivitis and its severe outcome: periodontitis.
This is a very powerful antiseptic that does not cause resistance and whose toxicological profile is favourable. But there is more: it is also known for its unique quality: SUBSTANTIVITY.
This puzzling term describes the capacity of our CHX to "stick" to structures in the mouth and to be released little by little, therefore ensuring an effect that is sustained over time.

In short, we can compare CHX's properties with those of another well-known antiseptic that is used in oral care: Cetylpyridinium Chloride (CPC):

PROPERTIES	CHX	CPC
HIGH ANTISPETIC ACTIVITY	YES	YES
HIGH ANTIPLAQUE POWER	YES	YES
DOES NOT CAUSE RESISTANCE	YES	YES
SUBSTANTIVITY*	YES (8-12 hours)	YES (3-4 hours and only if its concentration is 0.05 %)

Fig. 5

(*): ability of the molecule to bind to structures in the mouth for a time during which it is progressively released.

The mechanism of action is practically the same for both antiseptics: rupture of the cell wall resulting in cytoplasm leakage and then cell death.

We can see that in the "odd couple" CPC also has first order properties but with less substantivity.

Here is its structure:

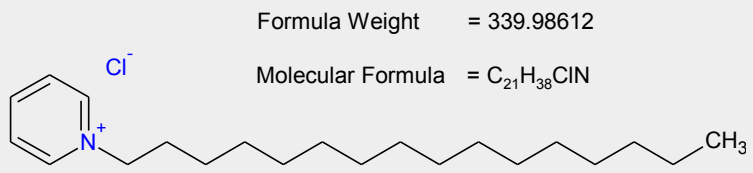


Fig. 6

As we can see also in this case, this is a quite voluminous molecule, with a permanently positive charge in the Nitrogen atom of the pyridinic nucleous: this positive charge along with the long "tail" (15 units methylene $–CH_2-$ + a terminal $–CH_3$) make up the typical structure of a surfactant and are responsible for its high antiseptic activity.

Based on the above, it can be concluded that CHX and CPC molecules offer powerful antibacterial activity that allows us to effectively treat and prevent periodontal and peri-implant diseases.

It is important to notice that adding these ingredients to formulas is not easy if we want to achieve MAXIMUM EFFICACY in fighting these diseases.

Despite their favourable physicochemical characteristics (i.e. high solubility in water), simply dissolving theses antiseptics in any excipient is not enough to yield a product with elevated antiseptic and anti-plaque activity.

In fact:

a. These two molecules require in depth study and selection of adequate excipients for the formula to which they will be added, because it can be easy to inactivate them
b. We must be able to carefully assess the activity of the formulated product

INACTIVATION of an antiseptic or any other active substance can be defined as a phenomenon or set of phenomena that prevents the expected therapeutic action from occurring with the desired strength and in the way needed.

How can we measure the activity of product formulated with antiseptics?

There are 3 methods:

i. ***In vitro* testing on a set of microorganisms present in oral flora:** consists of placing a bacterial suspension in contact with the product containing the antiseptic for 1 minute. Subsequently, the amount of survivor microorganisms is assessed. This Test is known as the SIKT (Short Interval Killing Test), which was developed by DENTAID® and published in the Journal of Clinical Periodontology in 2003. The purpose of this Test is to get an initial idea of the tested product's activity, and it is a very valuable tool in the development stage of new formulations.

ii. ***In vitro* test on BIOFILMS containing microorganisms that are present in the oral cavity:** the test product is applied for 1 minute to a group of bacteria in the form of a biofilm, a very complex structure, made up of layers of bacteria, that adheres to a surface (normally Hydroxyapatite discs) and where bacteria are embedded in polysaccharide elements that protect them. This type of test more closely simulates reality, and the bacteria are not as vulnerable as when they are found in suspension. The outcome of this Test is very important for accurately evaluating the product's potential: the antiseptic activity of two products that have yielded the same or a similar result in the SIKT can be effectively differentiated with this test. The technology required for testing the antiseptic activity on BIOFILMS is, however, VERY COMPLICATED and requires greater processing time. At DENTAID® we generate BIOFILMS that are identical to those present in the mouth with some 10 different bacterial species, using a specific bioreactor and an ARTIFICIAL MOUTH which has been the focus of numerous scientific studies. Once the biofilm has been in contact with the test product, specific dyes are applied and CONFOCAL MICROSCOPY is used to analyse the results obtained.

iii. **Standard or multicenter clinical trials:** clinical studies that are conducted by different research groups that test the product simultaneously and independently, on patients with different oral diseases.

In an upcoming chapter we will carefully study the role of the excipients in these formulations.