DRUG DELIVERY SYSTEMS BASED ON INORGANIC MATRIX

MASTER THESIS

CHEMICAL ENGINEERING

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1. Introduction

Clinicians historically have attempted to direct their interventions to areas of the body at risk or affected by a disease.
Depending on the drug and, the way it is delivered, and how our bodies respond, side effects sometimes occur.

Administering the drug systematically will produce more side effects because of it affects the whole the body. Administering the drug locally decreases the side effects and also maximizes the treatment's impact, making it more effective. [19].

The need to administer drugs only in the tissues or organs affected has made drug delivery so important in the medical field and that's why it has evolved so much over the last decades.

The evolution began with the first generation of drug delivery (1950-1980) which was focused on oral and transdermal release systems and establishing controlled drug release mechanisms. The second generation (1980-2010) was dedicated to the development of zero-order release systems, self-regulated drug delivery systems, long-term depot formulations, and nanotechnology-based delivery systems. [20].

2. Purpose and scope

The main objective of the thesis is to develop drug delivery systems based on inorganic matrix. To achieve this goal, the first step is synthesizing the composites based on two amino acids: histidine and poly [(R)-3-hydroxybutyric acid]. The second step is select the best composites from the two series and then modify them with the drug, clindamycin.

The steps followed in this project are:

- The synthesis of composites based on histidine and on poly [(R)-3-hydroxybutyric acid].
- The preparation of the solutions used during studies:
    - Ringer's liquid solution
    - Artificial saliva
    - Simulated body fluid
- Carry out the incubation and swelling studies with the composites prepared before.
- The preparation of the composites again containing clindamycin.
- Carry out the swelling studies with the samples containing clindamycin.

3. Theoretical part

3.1. Drug delivery systems

A drug delivery system (DDS) is the method of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals.

The whole process includes the administration of the therapeutic product, the release of the active ingredients by the product and the posterior transport of the active ingredients to the site of action.

Some advantages of drug delivery systems over traditional systems are the ability to deliver the drug more selectively to a specific site; easier, more accurate, less frequent dosing. Also it's possible to improve the efficacy and safety because of the capability of controlling the rate and time of release of the drugs in the body.

3.1.1. Drug delivery routes

Depending on the disease and the effect desired one or another route of administration will be chosen. Drugs may be administered directly to the organ affected.

A classification of various anatomical routes is shown in the table below.

Table 1. Classification of some drug delivery routes

Gastrointestinal system	Oral
	Rectal
Parenteral	Subcutaneous injection
	Intramuscular injection
	Intravenous injection
	Intra-arterial injection
Transmucosal: buccal an through mucosa lining the rest of gastrointestinal tract	
Transnasal	
Pulmonary: drug delivery by inhalation	
Transdermal drug delivery	
Intra-osseous infusion	

[1], [2], [3].

3.1.2. Delivery vehicles

Drug delivery vehicles are passive devices functioning mainly through a diffusion process in which the release of drugs is controlled either by the rate of diffusion through the pores of the drug carriers or by the rate degradation of the carrier matrices.

An ideal drug delivery vehicle must be non-toxic, biocompatible, non-immunogenic, biodegradable and must avoid recognition by the host's defense mechanisms.

There are different types of drug delivery vehicles such as:

- Liposomes

  Liposomes are the most common vehicle currently used for targeted drug delivery. They are composite structures made of phospholipids and may contain small amounts of other molecules.

  Liposomes are non-toxic, non-hemolytic, non-immunogenic, biocompatible, biodegradable and can be designed to avoid clearance mechanisms.

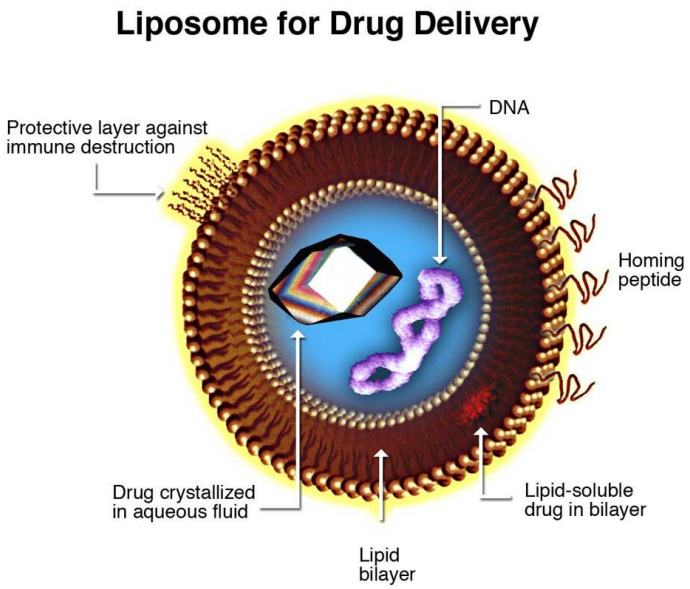


Figure 1. Schema of a liposome for drug delivery

- Polymeric micelles

  Polymeric micelles are self-assembled core-shell nanostructures formed in an aqueous solution consisting of amphiphilic block copolymers.

Polymeric micelles can be used to carry drugs that have poor solubility and they present a great potential as a drug delivery system for compounds that are hydrophobic and exhibit poor bioavailability which results from the unique core-shell structure.

- Biodegradable particles

Biodegradable particles are able to focus on the affected tissue and also deliver their payload as a controlled-release therapy. [4], [5], [6].

- Artificial DNA nanostructures

Recently, there have been developed two methods for building nanostructures using DNA which can be used in some drug delivery applications delivering therapeutics in a programmable way.

  - One of the techniques is called "DNA-brick assembly". This method uses short, synthetic strands of DNA that work like interlocking Lego® bricks, permitting to program DNA to form into predesigned shapes due to the base pairs; Adenosine only binds to Thymine and Cytosine only binds to Guanine.
  - The other technique is DNA origami. Using the principle of programmable self-assembly, strands of DNA are directed to form custom, specific shapes of tightly cross-linked double helices. [7].

3.1.3. Targeting strategies

Targeting methods allow nanoparticles to concentrate only in areas of diseased tissue. There are two kinds of targeting strategies: passive and active.

- Passive targeting

Passive targeting is accomplished covering up the nanoparticle with some kind of coating and the drug's success is directly related to the circulation time. Adding the coating substance to the surface of the nanoparticle, it becomes hydrophilic so the water molecules can bind to the oxygen molecules of the coating substance via hydrogen bonding. Due to this bond, a film of hydratation is formed around the nanoparticle making the substance antiphagocytic.

- Active targeting

Active targeting increases the effects of passive targeting making the nanoparticle more specific to a target site. There are some kinds of active targeting, such as:

  - Knowing the nature of a receptor on the cell for which the drug will be targeted to.
  - Utilizing magnetoliposomes and grafting them with a drug to deliver to a region of the body.
  - Utilizing materials that are pH responsive because a nanoparticle could have the capability to be activated by a trigger that is specific to a target site. Utilizing the pH can be effective because most of the body has a neutral pH and some parts of it are more acidic than others so nanoparticles can take advantage of this releasing the drug when it meets a specific pH.
  - Basing on redox potential. One of the effects of tumors is hypoxia which alters the redox potential in the area of the tumor. Modifying the redox potential that triggers the payload release, the vesicles can be selective to different types of tumors.

Utilizing both targeting methods; active and passive, a drug-loaded nanoparticle has a huge advantage over a conventional drug, because of it is able to circulate throughout the body for a long period of time until it is successfully attracted to its target through the types of active targeting previously commented. [4].

3.2. Histidine

Histidine is an essential amino acid and one of the 23 proteinogenic amino acids. Its chemical formula is $C_6H_9N_3O_2$ and its molar mass is 155.16 $g \cdot mol^{-1}$.

It contains an α-amino group, a carboxylic acid group and an imidazole functional group, a chain partially protonated, which classifies it as a positively charged amino acid at physiological pH.

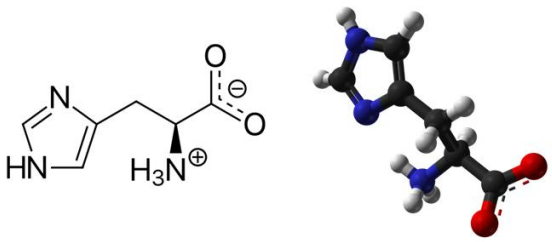


Figure 2. Skeletal formula and ball and stick model of histidine

L- histine is essential for both adults and childrens but, especially, childrens need an additional source of histidine that they can get through breast milk or special supplements to avoid a deficiency of L-histine that may cause growth problems or other conditions. It's also called essential because of is produced in very small amounts by the body. Histidine also helps for those who are recovering from an illness.

3.2.1 Functions of L-histidine

1. Histidine can be converted to different substances such as: histamine, glutamate and haemoglobin.
2. Ensures indirectly the oxygen supply to all the organs and tissues.
3. Ensures the energy supply in cells and can detoxify the body of heavy metals because is able to combine with them.
4. Regulates the pH of the blood.
5. A lack of histidine can lead to slow development and regeneration of tissue, inflammation of the skin and mucous and a slower operations or surgical procedures.
6. Necessary for the formation of myelin sheath, which surrounds all nerve cells protecting them from damage.
7. Can be used to prevent some degenerative diseases such as: Alzheimer and Parkinson.
8. L-histidine is involved in the synthesis of red and white blood cells, influencing the activity of the immune system.
9. Can protect the body from radiation, by binding itself to the damaging molecules and consequently eliminating them.
10. Can be used in case of inflammation, in the treatment of arthritis and help to reduce the symptoms of allergies. [8], [9].

3.3. Poly [(R)-3-hydroxybutyric acid]

Poly-3-hydroxybutyric acid, also known as polyhydroxybutyrate (PHB) is a polyhydroxyalkanoate (PHA).

Polyhydroxyalkanoates (PHAs) are bacterial polymers that are formed as naturally occurring storage polyesters by a wide range of microorganisms usually under unbalanced growth conditions.

The poly-3-hydroxybutyric acid is the most common form of polyhydroxyalkanoate.

PHB is a storage polyester occurring as insoluble inclusion bodies in the cytoplasm of a number of microbial species.

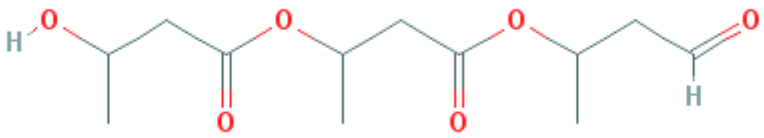


Figure 3. The 2D structure of PHB

PHB is produced by microorganism such as: Eshericia coli, saccharomyces cerevisiae and bacillus megaterium.

3.3.1 Advantages and disadvantages

- Advantages

  - Biodegradability

In the one hand, the biodegradation of plastics produced from petrochemicals is estimated to be around 400-600 years due to it's difficult to break up the long chains of molecules.

On the other hand, PHB can be degraded by microorganisms to carbon dioxide, methane and water reducing the environmental impact.

  - Non-toxicity properties

PHB is non-toxic and biologically inert.

  - Biocompatibility

Thanks to this property, PHB can be used in some medical applications such as: surgical suture, biodegradable screws for cartilague and bone fixation, biodegradable membranes and surgical meshes.

  - Produced from renewable resources

PHB is derived from natural sources of carbon such as glucose.

- Disadvantages

PHB is more expensive than petrochemical based polymers and this fact limits PHB based plastics to some medical applications. [15], [16], [17].

Figure 4. PHB granules

3.4. Clindamycin

Clindamycin is an antibiotic useful to treat bacterial infections in the body. It's also known as Cleocin, Dalacin and Clinacin.

The skeletal structure and the ball and stick model are de following ones:

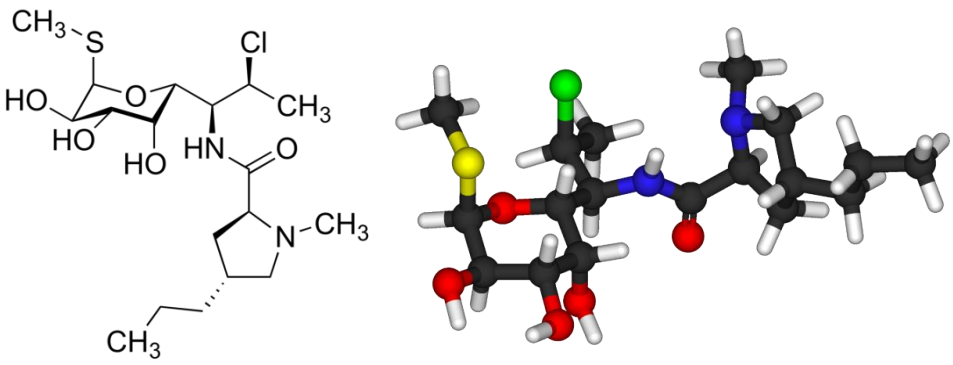


Figure 5. Skeletal formula and ball and stick model for Clindamycin

3.4.1. Medical uses

As commented before, clindamycin is used to treat anaerobic infections caused by anaerobic bacteria. Some of the diseases that can be treated with clindamycin include dental infections, infections of the respiratory tract, skin, soft tissue and peritonitis.

The topical way of administration of clindamycin phosphate can be used also to treat acne.

In people with hypersensitivity to penicillin, this drug can also be used for infections caused by aerobic bacteria.

Clindamycin it's also used to treat bone and joint infections, normally the ones caused by *Staphylococcus aureus.*

Malaria can also be treated with this drug but it must be given with chloroquine or quinine. Clindamycin should not be used as an antimalarial by itself although is very effective due to its slow action. [10,11].

3.4.2. Routes of application

The routes of administration of the drug are: oral, topical, intravenous and intravaginal.

- The topical application is referred to the medication that is applied in a particular place of the body.
- The intravenous therapy is the infusion of liquid substances directly into the veins. The intravenous route is the fastest way to deliver fluids and medications throughout the body.
- The intravaginal way of administration is achieved through a pessary, a medical device inserted into the vagina. [10],[13],[14].

3.4.3. Mechanism of action

Clindamycin has primarily bacteriostatic effect. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation so it works primarily by binding to the 50s ribosomal subunit of bacteria.

3.4.4. Side effects

The side effects can be divided in two groups:

- Common side effects
    - Stomach pain
    - Nausea
    - Vomiting
    - Diarrhea
    - Rash
    - Metallic or unpleasant taste in the mouth

- Serious side effects
    - Antibiotic-associated diarrhea. The symptoms can include:
        - Severe diarrhea
        - Bloody diarrhea
        - Stomach cramping and pain
        - Fever
        - Dehydratation
        - Loss of appetite
        - Weight loss
    - Severe skin rashes and toxic epidermal necrolysis. The symptoms can include:
        - Severe rash
        - Peeling skin
        - Swollen face or tongue
        - Blisters on your skin or blisters in or around the nose, mouth and eyes. [12].

4. Experimental part

4.1. Synthesis of composites

Firstly, aqueous solutions of PVP (polyvinylpyrrolidone), Histidine or PHB (poly [(R)-3-hydroxybutyric acid]) and gelatine were prepared and then mixed to prepare the different samples.

The concentrations required for the aqueous solutions are:

Table 2. Concentrations required for aqueous solutions

Solution	Concentration
PVP	15 %
Histidine or PHB	0.5 %
Gelatin	2 %

Two series will be prepared which will have the same compositions of: the aqueous solutions prepared previously (except the amino acid), the crosslinking agent and the photoinitiator and only the amino acid will be changed.

The amino acid of the first serie will be histidine and the amino acid of the second serie will be poly [(R)-3-hydroxybutyric acid].

The compositions of the composites are the following ones:

Table 3. Compositions of composites on the basis of histidine

SERIE 1					
Sample	**PVP (ml)**	**Histidine (ml)**	**Gelatin (ml)**	Crosslinking agent **PEGDA(*) (ml)**	Photoinitiatior **2-hydroxy-2-methylpropiophenone (ml)**
1	1	5	4	1.6	0.25
2	2	4	4	1.6	0.25
3	3	3	4	1.6	0.25
4	4	2	4	1.6	0.25
5	5	1	4	1.6	0.25

(*) PEGDA. Polyethylene (glycol) diacrylate.

Table 4. Compositions of composites on the basis of poly [(R)-3-hydroxybutyric acid]

SERIE 2					
Sample	**PVP (ml)**	**Poly [(R)-3-hydroxybutyric acid] (ml)**	**Gelatin (ml)**	Crosslinking agent **PEGDA (ml)**	Photoinitiatior **2-hydroxy-2-methylpropiophenone (ml)**
1	1	5	4	1.6	0.25
2	2	4	4	1.6	0.25
3	3	3	4	1.6	0.25
4	4	2	4	1.6	0.25
5	5	1	4	1.6	0.25

To prepare each composite, previously PVP, histidine or poly [(R)-3-hydroxybutyric acid] and gelatine should be mixed. Then, once the lamp is turned on, the crosslinking agent and the photoinitiator are added to the mixture and this one, placed on a petri dish. After that, the petri dish is also placed under the lamp until the mixture is solid.

Once the composites are ready, they must be cutted into different forms and wait for them to get dry for subsequent swelling studies.

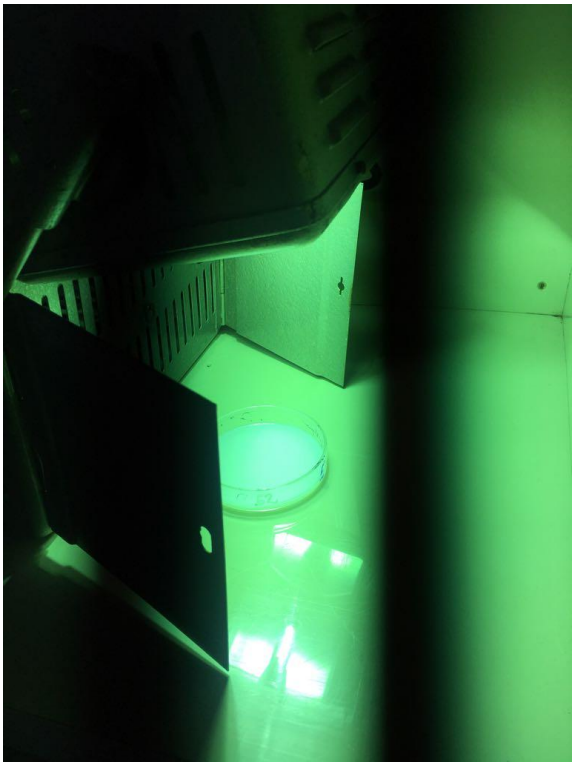


Figure 6. Sample under the lamp

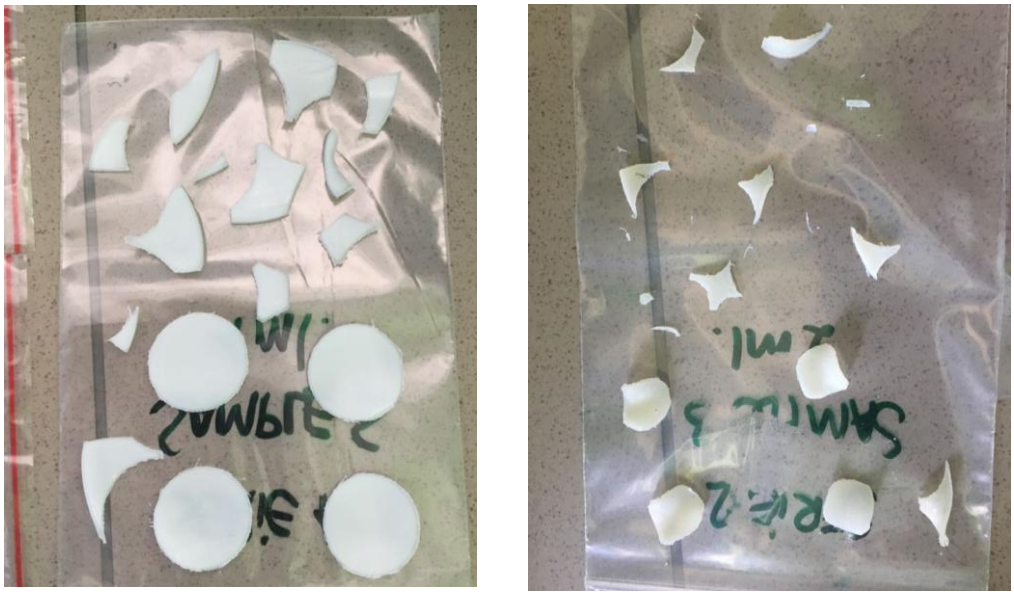


Figure 7. Sample after being cutted and dry sample

4.1.1. Studies on prepared materials

4.1.1.1. Preparation of solutions used during studies

4.1.1.1.1. Ringer's liquid solution

The ringer's solution is a solution of salts dissolved in water for the purpose of creating an isotonic solution relative to the body fluids.

Ringer's solution is frequently used in in vitro experiments on organs or tissues. [18].

The volume required of this solution is 1 liter. The components and quantities needed to prepare 1 liter of it are collected in the following table.

Table 5. Components and quantities needed to prepare 1L of Ringer's liquid solution

Component	Quantity (g)
NaCl	8.6
KCl	0.3
$CaCl_2$	0.48

The components should be mixed in the order shown in the table. It's important to comment that the next ingredient must be added after the complete dissolution of the previous one.

4.1.1.1.2. Artificial saliva

The volume needed for this solution is the same as for Ringer's solution, 1 liter. To prepare the volume required, the components and quantities are:

Table 6. Components and quantities needed to prepare 1L of Artificial saliva

Component	Quantity (g)
NaCl	0.4
KCl	0.4
$CaCl_2 \cdot H_2O$	0.755
$NaH_2PO_4 \cdot H_2O$	0.780
$Na_2S \cdot 5\ H_2O$	0.005
Urea	1

Also can be prepared with $CaCl_2$ and NaH_2PO_4 instead of $CaCl_2 \cdot H_2O$ and $NaH_2PO_4 \cdot H_2O$. The quantities used with these components are the following ones:

Table 7. Components and quantities needed to prepare 1L of Artificial saliva

Component	Quantity (g)
NaCl	0.4
KCl	0.4
$CaCl_2$	0.684
NaH_2PO_4	0.678
$Na_2S \cdot 5\ H_2O$	0.005
Urea	1

The procedure to prepare this solution is the same as the one to prepare the ringer's liquid solution. The next ingredient must be added after the complete dissolution of the previous one.

4.1.1.1.3. Simulated body fluid

The volume required of this solution is 2 liters and the conditions of this solution are 36.6°C and pH 7.4.

The components must be added in the following order and to add the next ingredient it's important to wait until the previous one is completely dissolved (the same as in the previous solutions).

Table 8. Components and quantities needed to prepare 2L of Simulated Body Fluid

Component	Quantity (g)
NaCl	16.07
$NaHCO_3$	0.710
KCl	0.450
$K_2HPO_4 \cdot 3\ H_2O$	0.462
$MgCl_2 \cdot 6\ H_2O$	0.622
HCl 1M	78 ml
$CaCl_2$	0.584
Na_2SO_4	0.144
TRIS	12.236

4.1.2. Incubation studies

To carry out with the incubation studies the samples must be prepared. For this preparation, the samples prepared previously (5 in each serie) must be introduced into the solutions prepared before; Ringer's liquid solution, Artificial saliva, Simulated body fluid and, also, distilled water, so 40 samples will be prepared (20 samples of each serie). After this, the pH of each sample will be measured.

Incubation studies will be performed at room temperature.

4.1.3. Swelling studies

Swelling studies are based on the weight difference between the samples before and after introducing them into the solutions.

The samples prepared will be the same as the ones used for incubation studies.

Before introducing the sample into the solution, each one must be weighed and after the samples has been submerged for a time (1 hour, 48 hours) this ones will have to be weighed again to measure the weight difference.

After all the measurements have been done, to continue with the swelling studies it's necessary to calculate the swelling ratio. The swelling's ratio formula is:

$$Q = \frac{w - w_0}{w_0}$$

Where:

Q – Swelling ratio (g/g)

w – weight of swollen sample (g)

w_0 – hydrogel weight before immersing in solution (g)

Swelling studies are important because of calculating the swelling ratio it's possible to know which are the best samples in releasing the drug. The highest swelling ratio is traduced in the best release of the drug.

4.2. Results of studies

4.2.1. Incubation studies

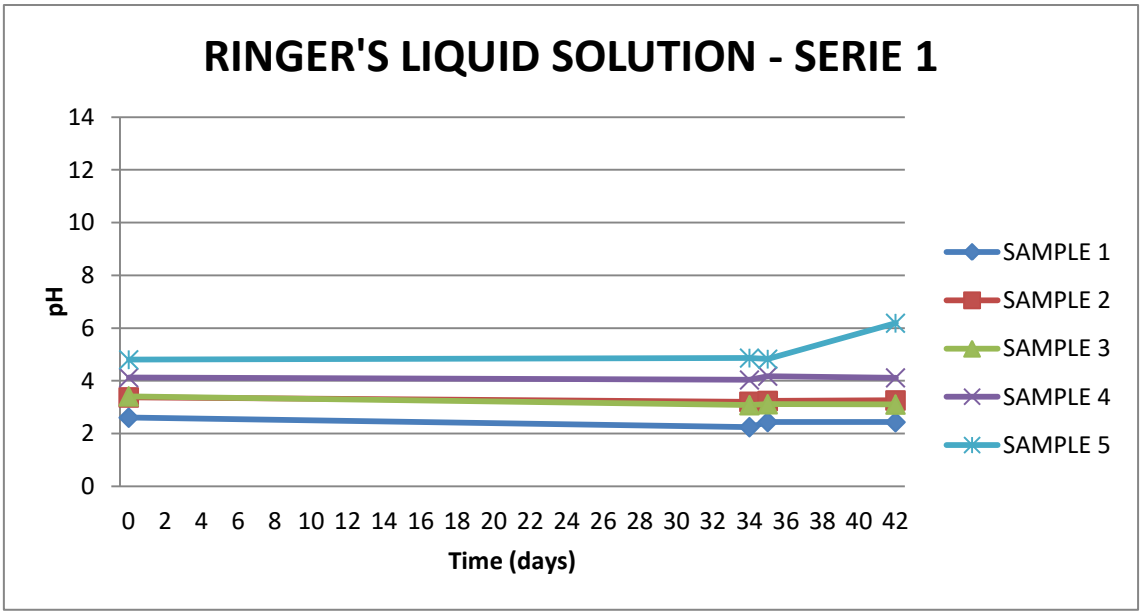
During the incubation studies, four pH measurements have been done during 42 days.

The graphs shown below belong to the first serie of samples, the serie based on histidine. The composition of composites of this serie, as commented before, is:

Table 9. Composition of composites of Serie 1

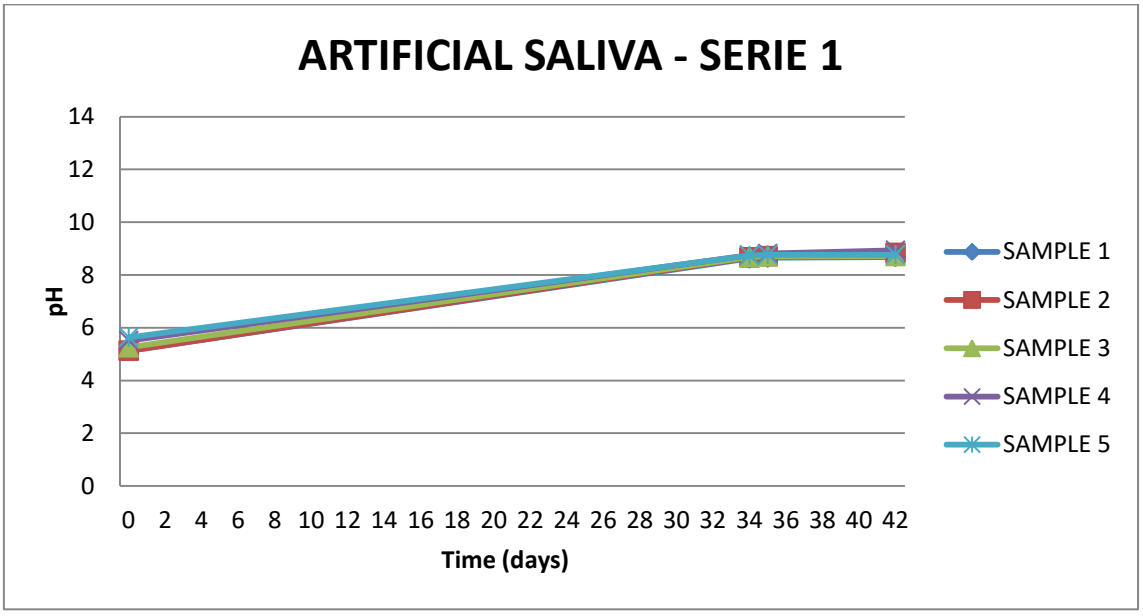
SERIE 1		
Sample	**PVP (ml)**	**Histidine (ml)**
1	1	5
2	2	4
3	3	3
4	4	2
5	5	1

The graphs are the following ones:



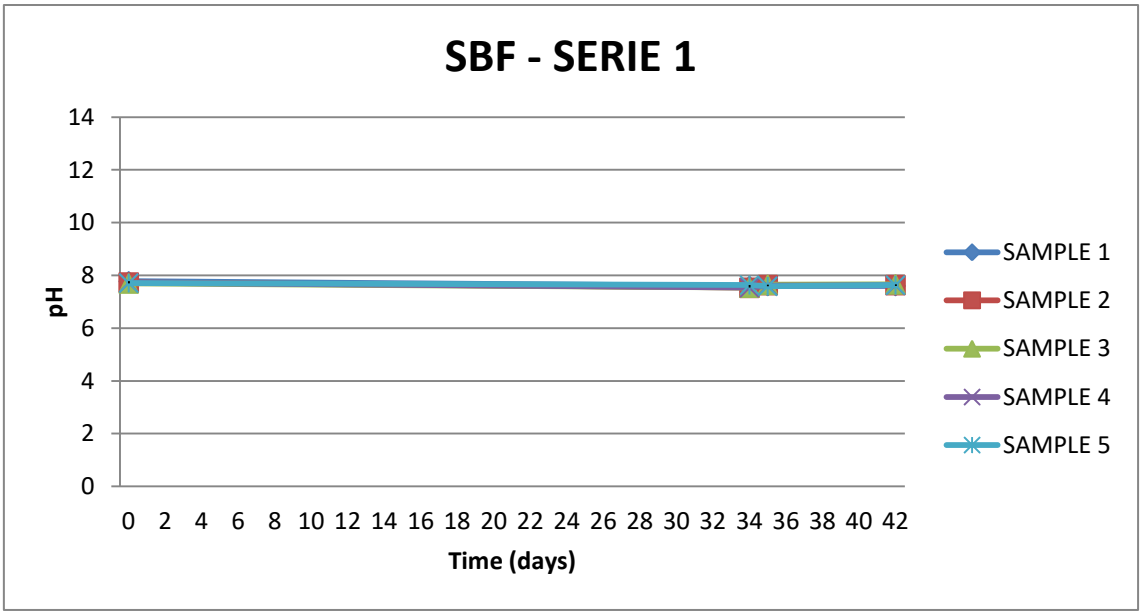
Graph 1. Incubation studies results of Serie 1 for Ringer's liquid solution

It can be observed that the general tendency in this graph is to maintain a constant pH in an acid environment.



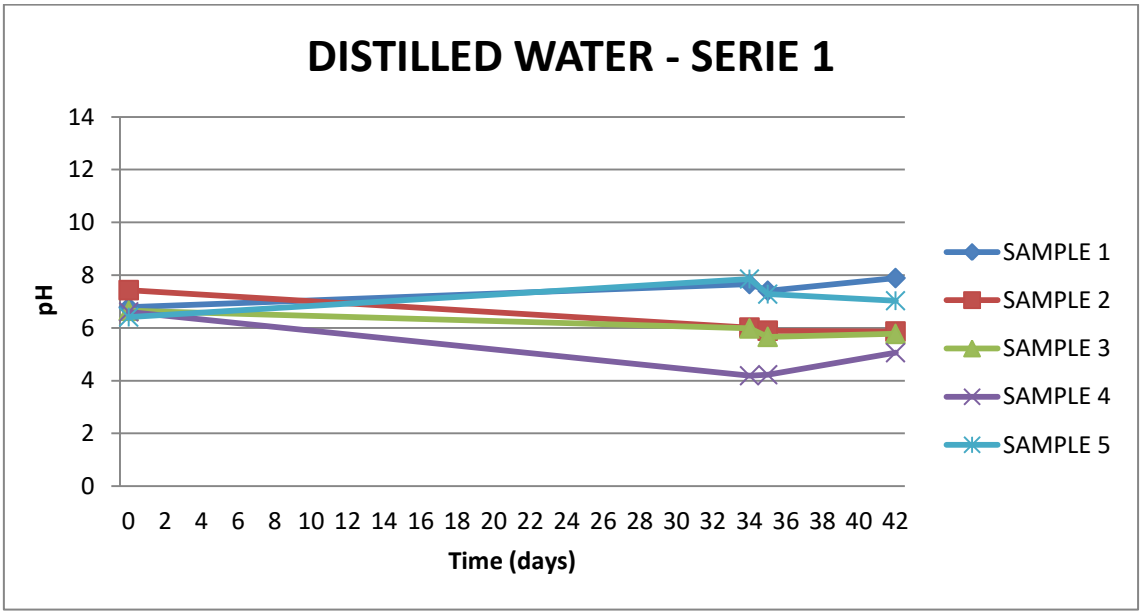
Graph 2. Incubation studies results of Serie 1 for Artificial saliva

The pH increases gradually during the first month and then stabilizes during the last three measurements.



Graph 3. Incubation studies results of Serie 1 for Simulated Body Fluid

The pH is constant throughout the incubation study.



Graph 4. Incubation studies results of Serie 1 for Distilled water

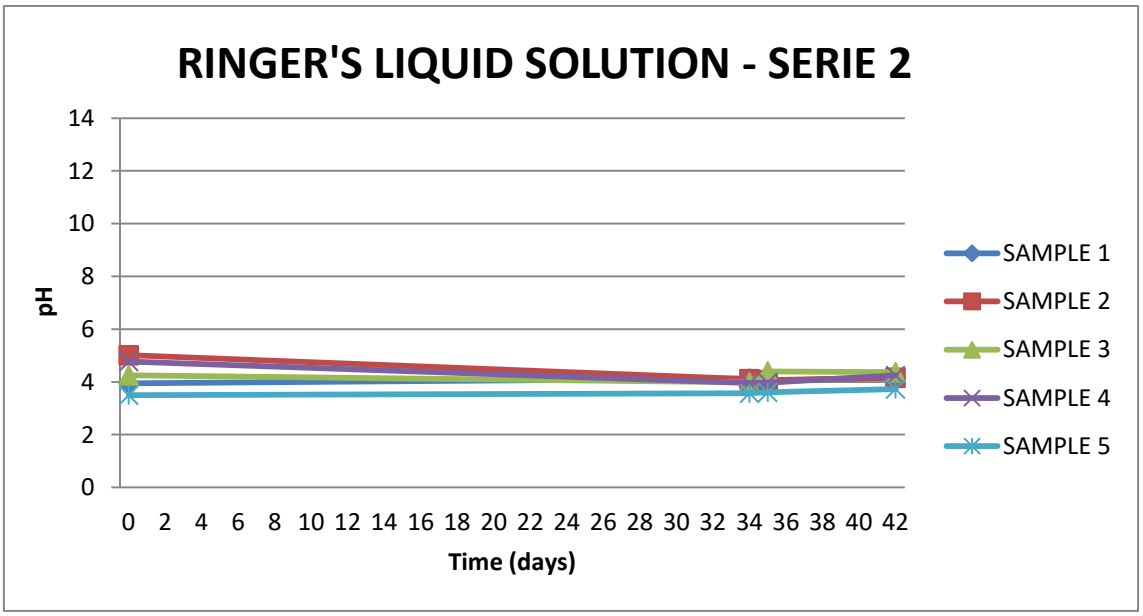
It is in the solution in which the values of ph oscillate more. There are some rapid changes in the values of pH of the different samples.

The composition of composites of the second serie, the one based on poly [(R)-3-hydroxybutyric acid]), as commented before, is:

Table 10. Compositions of composites of Serie 2

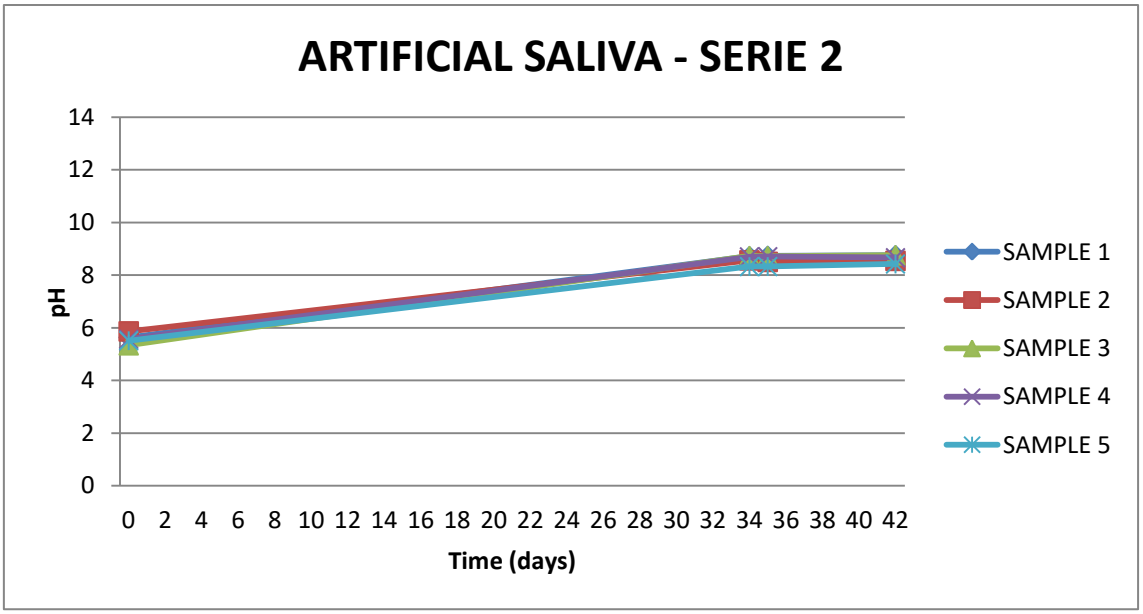
SERIE 2		
Sample	**PVP (ml)**	**Poly [(R)-3-hydroxybutyric acid] (ml)**
1	1	5
2	2	4
3	3	3
4	4	2
5	5	1

The graphs belonging to the second serie are the following ones:



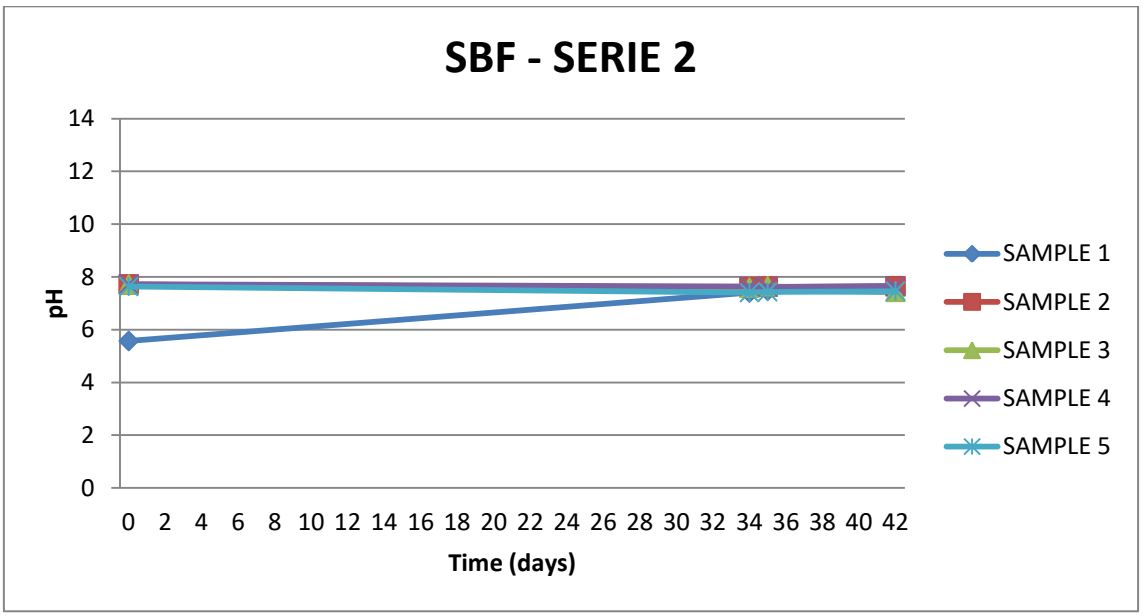
Graph 5. Incubation studies results of Serie 2 for Ringer's liquid solution

As for series 1, the general trend is constant in an acid environment.



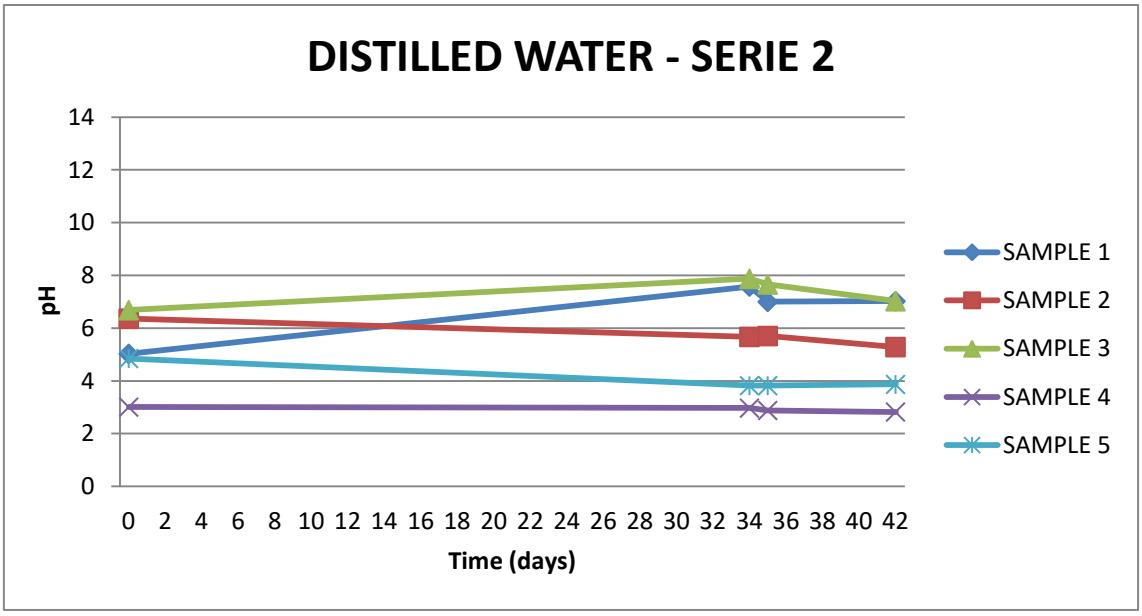
Graph 6. Incubation studies results of Serie 2 for Artificial saliva

As for series 1, the pH increases gradually during the first month and then stabilizes during the last three measurements.



Graph 7. Incubation studies results of Serie 2 for Simulated Body Fluid

For this series the general trend is also constant ph.



Graph 8. Incubation studies results of Serie 2 for Distilled water

As for series 1, the values of pH oscillate much between one sample and another and also, there are some rapid changes between the values of the same sample during the days of the incubation study.

4.2.2. Swelling studies

Measurements during swelling studies were performed after 1 hour and 48 hours.
In the graphs shown below it is represented the swelling ratio (Q) for each sample of each solution. There are four graphs for the four solutions with which the studies have been developed.
The composition of the composites for the serie based on histidine, serie 1 is:

Table 11. Composition of composites of Serie 1

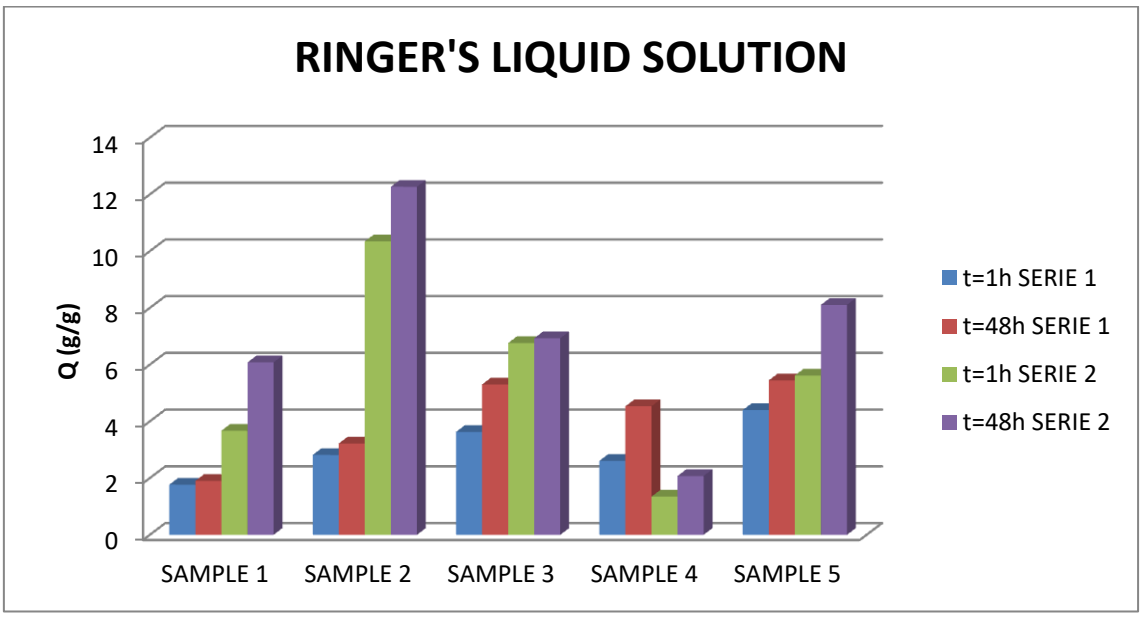
SERIE 1		
Sample	**PVP (ml)**	**Histidine (ml)**
1	1	5
2	2	4
3	3	3
4	4	2
5	5	1

The composition of composites for the serie 2, based on poly[(R)-3-hydroxybutyric acid]), is:

Table 12. Composition of composites of Serie 2

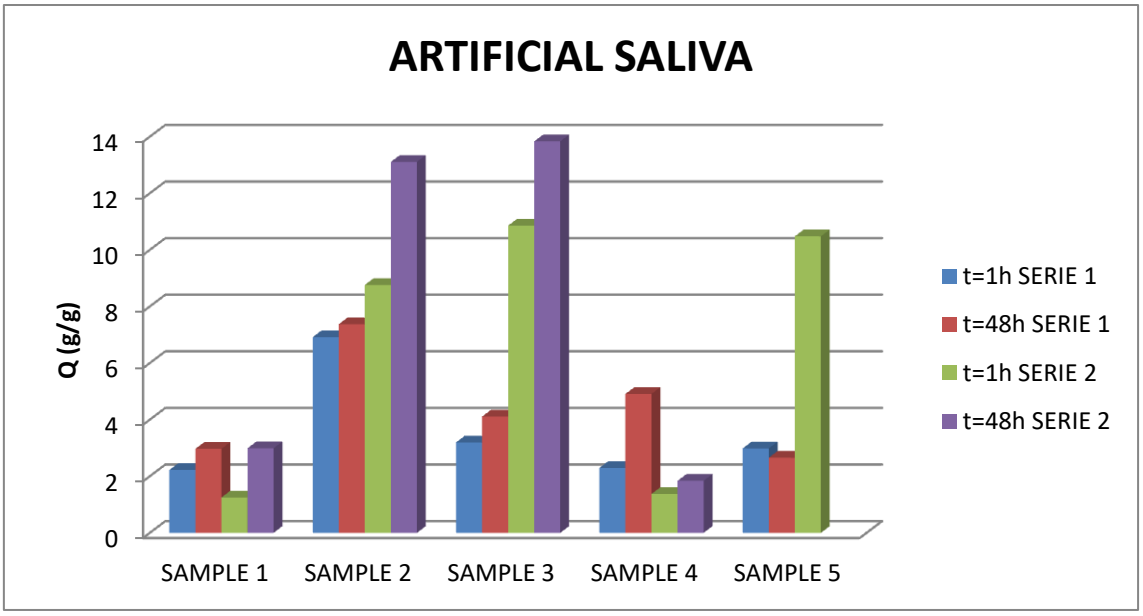
SERIE 2		
Sample	**PVP (ml)**	**Poly [(R)-3-hydroxybutyric acid] (ml)**
1	1	5
2	2	4
3	3	3
4	4	2
5	5	1

The graphs are the following ones:



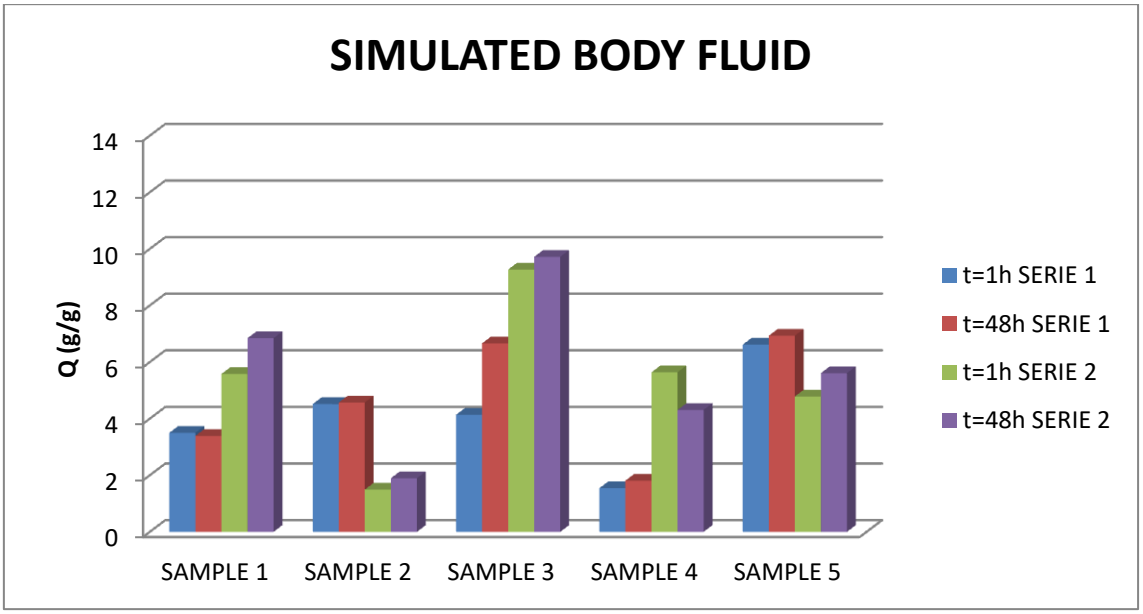
Graph 9. Swelling studies results for Ringer's liquid solution

The columns that have been taken into account are the ones with t=1h for both series 1 and 2. It can be observed that the highest values of Q are obtained for the sample 5 from serie 1 and the sample 3 from serie 2.



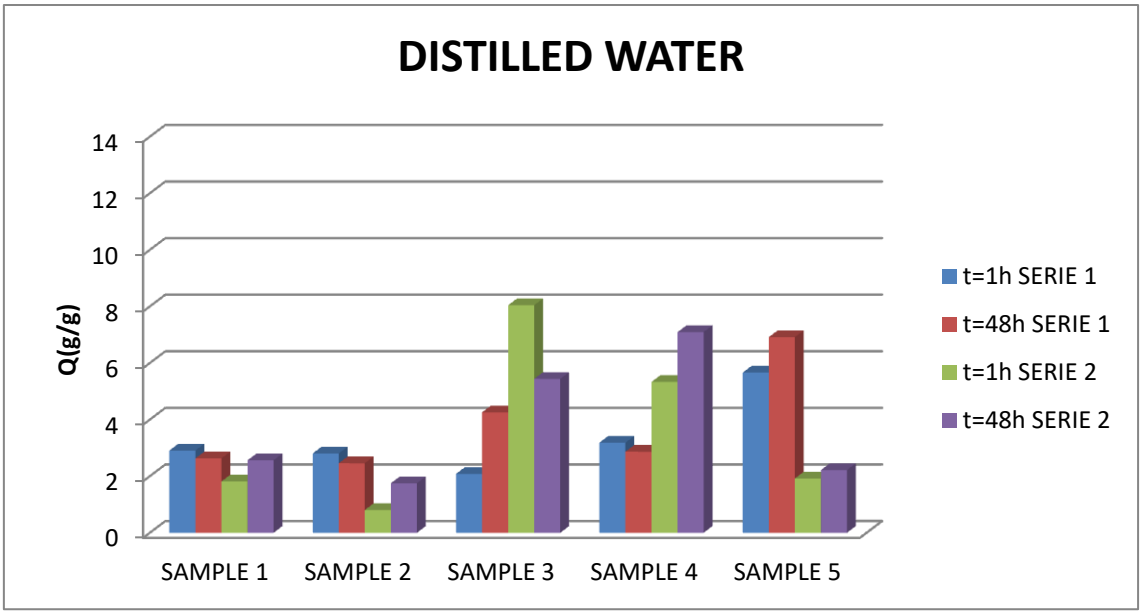
Graph 10. Swelling studies results for Artificial saliva

In this case, the highest swelling ratio is obtained for the sample 2 from serie 1 and the sample 3 from serie 2.



Graph 11. Swelling studies results for Simulated Body Fluid

The highest swelling ratio is obtained for the sample 5 from serie 1 and the sample 3 from serie 2.



Graph 12. Swelling studies results for Distilled water

The highest swelling ratio is obtained for the sample 5 from serie 1 and the sample 3 from serie 2.

The trend is clear. The samples with the highest swelling ratio are sample 5 from series 1 and sample 3 from series 2 so these ones are the ones chosen to be modified with the drug, clindamycin.

4.3. Synthesis of composites containing clindamycin

4.3.1. Preparation of composites

The solutions needed to prepare the composites are the same as the ones of the previous studies (PVP, gelatine, histidine and poly [(R)-3-hydroxybutyric acid]) and, also, a solution of diluted clindamycin.

The preparation of the diluted solution of clindamycin is carried out by diluting half tablet of clindamycin of 600 mg in 50 ml.

To carry out the preparation of composites it's important to take into account the result of the swelling studies done previously because of those samples with the best or highest swelling ratio will be chosen to carry out the synthesis of composites containing clindamycin because of, as it was commented before, the highest swelling ratio is traduced in the best release of the drug.

The samples with the highest swelling ratio are the Sample 5 for Serie 1 and the Sample 3 for Serie 2. Three samples of each sample will be prepared with different volumes of the solution of diluted clindamycin.

The compositions of composites required are the following ones:

Table 13. Compositios of composites of Serie 1 containing clindamycin

SERIE 1						
SAMPLE 5						
Sample	**PVP (ml)**	**Histidine (ml)**	**Gelatin (ml)**	Crosslinking **agent PEGDA (ml)**	Photoinitiator **2-hydroxy-2-methylpropiophenone (ml)**	**Solution of clindamycin (ml)**
5.1	5	1	4	1.6	0.25	1
5.2	5	1	4	1.6	0.25	2
5.3	5	1	4	1.6	0.25	3

Table 14. Compositios of composites of Serie 2 containing clindamycin

SERIE 2						
SAMPLE 3						
Sample	**PVP (ml)**	**Poly [(R)-3-hydroxybutyric acid] (ml)**	**Gelatin (ml)**	Crosslinking agent **PEGDA (ml)**	Photoinitiator **2-hydroxy-2-methylpropiophenone (ml)**	**Solution of clindamycin (ml)**
3.1	3	3	4	1.6	0.25	1
3.2	3	3	4	1.6	0.25	2
3.3	3	3	4	1.6	0.25	3

The procedure to prepare the composites it is the same as the one described on *4.1 synthesis of composites*. The only difference is that also the diluted clindamycin must be added to the mixture at the same time as PVP, histidine or Poly [(R)-3-hydroxybutyric acid] and gelatine.

4.3.2. Studies

4.3.2.1. Swelling studies

The procedure to carry out the swelling studies containing clindamycin it's a little bit different from the swelling studies procedure done before.

The theoric concept is the same, based on the weight difference before and after introducing the samples into the solutions.

The studies, in this case, will be developed on two environments:

- Acid environment, representing the pH in some acidic parts of the body such as the stomach (pH 2).
- Basic environment, representing the pH of other more basic parts of the body such as the blood (pH approximately of 7.4) and the saliva (pH between 6.2 and 7.4).

The solutions needed are:

- Citric acid monohydrate solution which represents the acid environment because its pH is 2.
- Phosphate buffered saline solution which represents the basic environment because its pH is 7.4.

For the citric acid monohydrate solution the volume and concentration required are 200 ml and 2%.

The volume required of the phosphate buffered saline solution is 200 ml. To prepare it is just enough to dissolve one tablet in the volume required.

Once the samples and the solutions are prepared, the first step is to weight each sample before introducing them into the two solutions. Then, the samples must be introduced into the solutions and after 1 hour it must be weighted again.

4.3.3. Results of swelling studies

The graphs for the swelling studies containing clindamycin represent the swelling ratio (Q) for each sample of each serie. There are two graphs, one for the citric acid monohydrate solution and the other for phosphate buffered saline solution.

The compositions of composites for serie 1 and serie 2 are:

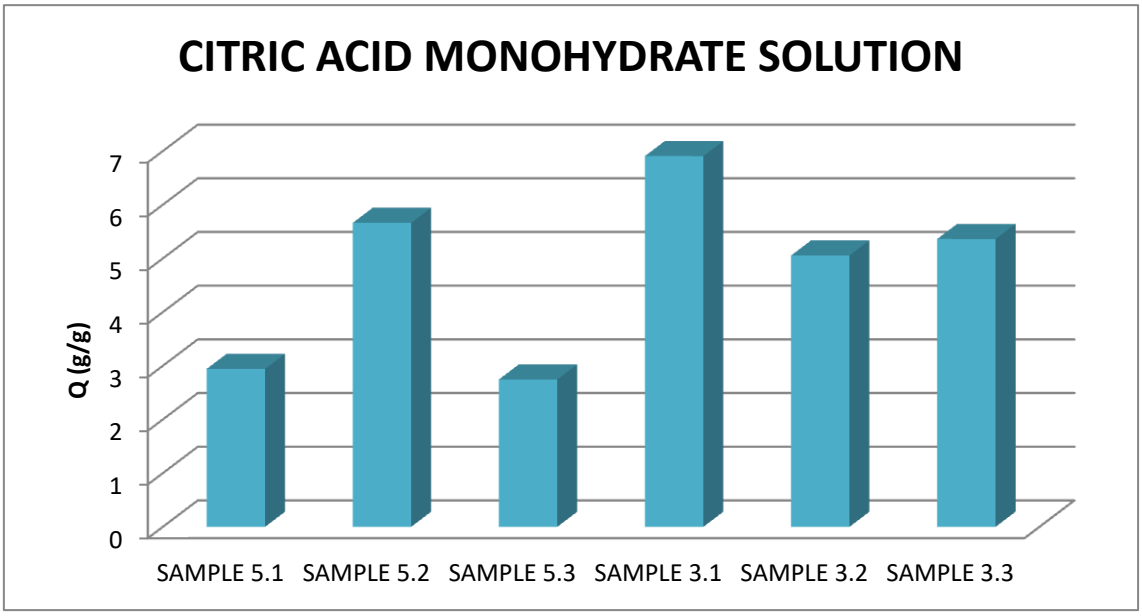
Table 15. Compositios of composites of Serie 1 containing clindamycin

SERIE 1				
SAMPLE 5				
Sample	PVP (ml)	Histidine (ml)	Gelatin (ml)	Solution of clindamycin (ml)
5.1	5	1	4	1
5.2	5	1	4	2
5.3	5	1	4	3

Table 16. Compositios of composites of Serie 2 containing clindamycin

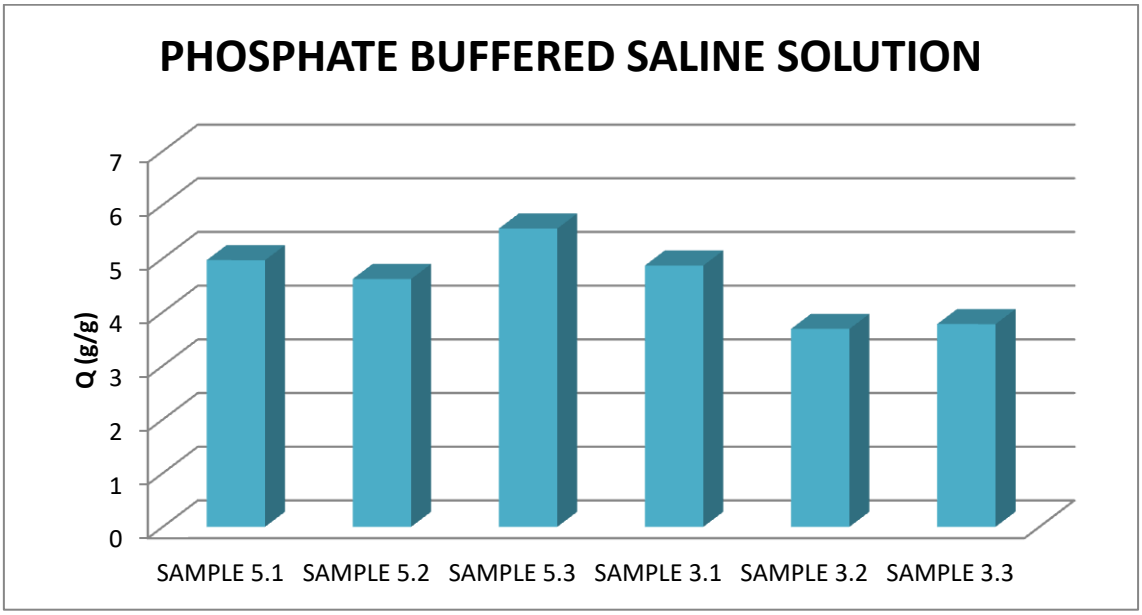
SERIE 2				
SAMPLE 3				
Sample	PVP (ml)	Poly [(R)-3-hydroxybutyric acid] (ml)	Gelatin (ml)	Solution of clindamycin (ml)
3.1	3	3	4	1
3.2	3	3	4	2
3.3	3	3	4	3

The graphs are the following ones:



Graph 13. Swelling studies results for Citric acid monohydrate solution

The samples with higher swelling ratio are the ones from the Serie 2, based on poly [(R)-3-hydroxybutyric acid]. These samples swell more in citric acid monohydrate solution, which means that the drug release will be better in an acidic environment.



Graph 14. Swelling studies results for Phosphate buffered saline solution

The samples from the Serie 1, the one based on histidine, are the ones which swell more in this solution so are characterized by higher swelling ability in alkaline environment and the drug release will be better in that conditions.

5. Conclusion

The conclusion to be taken from the results obtained during the incubation studies is that the general trend of the samples is to maintain a constant pH, except in the case of incubation studies in distilled water where the values of pH oscillate a little bit more.

In the case of the samples which maintain a constant value of pH, what can be interpreted is that the material is biocompatible with the tested solution; it means that there is no negative impact of the sample on the solution and also, there is no negative impact of the solution on the sample.

In the case of the samples where any rapid changes occur (the pH oscillates more), what can be interpreted is that these oscillations can be caused by the sample's degradation in the solution.

During the swelling studies with hydrogel, when the liquid penetrates into the hydrogel, solvation of hydrophilic groups and consequently their dissociation can be observed.

For example, carboxyl group dissociates with formation of COO^- and H^+. H^+ go to the solution but COO^- from the neighbouring groups pushes each other resulting on free spaces between polymer chains get bigger and consequently more liquid can be absorbed. In a liquid with ions such as Ca^{2+} or Mg^{2+}, they combine two carboxyl groups (form additional bonds between polymer chains) and that's why less free space for the liquid can be observed. This explains why hydrogel swells more in distilled water than in solutions containing ions.

The conclusions that can be obtained for the swelling studies are confusing. The comparison of the swelling ratios between the different solutions of the samples that have been used to be modified with clindamycin (Sample 5 from Serie 1 and Sample 3 from Serie 2) leads to affirm that; the Sample 5 swells more in the simulated body fluid solution and the Sample 3 swells more in artificial saliva. None of them swells better in distilled water.

Also, as it has been commented during the thesis, swelling is directly related to the release of the drug. That's way those samples were selected to be modified with the drug, because are the ones with higher swelling ratios. The higher swelling ratio means the best release of the drug.

In case of the swelling studies of composites which were modified with clindamycin which can be observed is that the samples from the Serie 1, the one based on histidine, swell more in phosphate buffered saline solution so are characterized by

higher swelling ability in alkaline environment so drug release will be better in that conditions. In case of the Serie 2, the one based on poly [(R)-3-hydroxybutyric acid], what can be observed is the opposite situation, the samples swell more in citric acid monohydrate solution, which means that the drug release will be better in an acidic environment.

Also, can be observed that the amount of the drug is not related to the swelling ratio because of in the case of the samples 5.1, 5.2 and 5.3 from Serie 1, which swells more in the phosphate buffered saline solution, the swelling ratio is higher for the sample with the highest amount of drug (5.3 with 3 ml) but the relation between the swelling ratio and the amount of the drug it's not proportional. In case of the samples from the second serie, which swells more in citric acid monohydrate, also the relation between the swelling ratio and the amount of the drug it's not proportional.

6. Abstract

A drug delivery system is the method of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals.

To develop a drug delivery system based on an inorganic matrix, the first step is synthesizing the composites, in this case, two series based on two different amino acids; histidine and poly [(R)-3-hydroxybutyric acid].

The second step is select the best composite basing on two studies; incubation and swelling. After that, the best composites will be modified with the drug.

The study finishes performing again the swelling studies to the samples modified with the drug and determining which environment, acid (which is the pH of some parts as the stomach) or basic (which is the pH of the saliva or the blood), is the best for releasing the drug in the human body for each series of composites.

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