



HAL
open science

Optimization of the formulation of an original hydrogel-based bone cement using a mixture design

Océane Ly, Francine Monchau, Sébastien Rémond, Christine Lors, Alain Jouanneaux, Étienne Debarre, Denis Damidot

► **To cite this version:**

Océane Ly, Francine Monchau, Sébastien Rémond, Christine Lors, Alain Jouanneaux, et al.. Optimization of the formulation of an original hydrogel-based bone cement using a mixture design. *Journal of the mechanical behavior of biomedical materials*, 2020, 110, pp.103886. <10.1016/j.jmbbm.2020.103886>. <hal-02999177>

HAL Id: hal-02999177

<https://hal.science/hal-02999177v1>

Submitted on 15 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

OPTIMIZATION OF THE FORMULATION OF AN ORIGINAL HYDROGEL-BASED BONE CEMENT USING A MIXTURE DESIGN

Océane Ly^{1,2}, Francine Monchau^{1*}, Sébastien Rémond², Christine Lors², Alain Jouanneaux³, Étienne Debarre¹, Denis Damidot²

¹Univ. Artois, ULR 4515 - LGCgE, Laboratoire de Génie Civil et géo-Environnement, F-62400 Béthune, France.

²IMT Lille Douai, Univ. Lille, ULR 4515 –LGCgE, Laboratoire Génie Civil et géo-Environnement, département Génie Civil & Environnemental, 941 rue Charles-Bourseul, 59508 Douai, France.

³Institut des Molécules et des Matériaux du Mans (IMMM), UMR-CNRS n° 6283, Le Mans Université, avenue O. Messiaen, 72085 Le Mans, France.

* To whom the correspondence should be addressed:

Francine Monchau, e-mail : Francine.monchau@univ-artois.fr

Abstract

Highly swelling polymers, i.e. superabsorbent hydrogels, are hydrophilic, three dimensional networks that can easily absorb a significant amount water, fluid or drug. They are widely used in various applications such as foods, cosmetics, and medical devices.

Bone cements are used in orthopaedics as a filling biomaterial or as a **grout** enhancing the embedding of a prosthesis into bone **and fixation is achieved by mechanical interlock with metal or bone surfaces. Recently, hydrophilic bone cements have attracted the attention for bone tissue-engineering applications.**

Here a bone cement containing an acrylic hydrogel (HEMA) as a liquid phase and a blend of corn starch, cellulose acetate and bioceramic filler as a solid phase is investigated by means of a mixture design which is a special topic within statistical Design of Experiments (DoE). Output variables of interest, complex shear modulus, compressive modulus and swelling rate related to rheological, mechanical and swelling properties respectively, are measured for each cement formulation.

Applying the mixture design strategy enables to assess the impact of the three powder components on each variable of interest and to determine the optimal formulation in order to achieve the required properties of this HEMA-based bone cement, especially the rheology adapted to the desired clinical application, but also appropriate mechanical and swelling properties.

Keywords: Mixture design, Bone cement, 2-hydroxyethyl methacrylate, Optimization, Mechanical properties, Rheology, Swelling.

1. INTRODUCTION

Bone is a particular natural material which has the stunning ability to heal by its own when broken, through a series of biological events following molecular signalling pathways. However, this ability remains limited in cases of critical size defects, when natural grafts (autograft, allograft or xenograft) or synthetic grafts (biomaterial) are needed to fix the damage.

As life expectancy increases, malfunction or loss of tissue caused by injury or disease leads to reduce quality of life in many patients at significant economic cost. Bone defects are one of the leading causes of morbidity and disability in elderly patients.

Tissue engineering is one of the fields of action of regenerative medicine. The main objective is to assist the regeneration of tissues damaged by disease or trauma. Current research on biodegradable polymers is emerging in the field of bone tissue engineering. As an alternative to autologous bone grafts, natural polymers are one of the most attractive options mainly due to their performance to facilitate *in vivo* bone regeneration. Recently, injectable hydrogels have attracted the attention for bone tissue-engineering applications because they can replace implantation surgery with a minimally invasive injection method and can form any desired shape, to match irregular defects (Liu et al., 2017). For example, starch can be used in the form of bone cement, scaffolds or microparticles in numerous clinical applications (Carvalho et al., 2016).

The cement may also have the consistency of a dough and inserted directly into the bone defect by hand or spatula (Farrar et al., 2001; Ly et al., 2017). Hence the control of the rheological properties is capital according to the type of sought-after applications.

Mainly two types of bone cements are currently used for this application: calcium phosphate based bone cements and/or acrylic based bone cements. Calcium phosphate cements (CPCs) are used since the 1990s. They are hydraulic cements, meaning that they are made by mixing a calcium phosphate powder and an aqueous liquid with no need of another constituent. The curing of the cement is made possible by a reaction of dissolution and precipitation. The final product is an apatitic cement or a brushitic cement (Ginebra et al., 2012). Their main interest is that they resemble the mineral fraction of the bone, meaning that they are highly bioactive, interacting with the osteoblasts and osteocytes of the bone. Nevertheless, as ceramic materials, their mechanical properties are pretty poor.

Historically used cements are acrylic-based; mainly polymethyl methacrylate (PMMA) cements which have known a soaring use since 1958 thanks to Sir John Charnley for total hip replacement (Charnley, 1960).

Those cements are widely used, since they are relatively cheap, practical to handle, can be modelled *in situ*, have important mechanical properties, and are not harmful to the body when fully cured. However, despite their popularity, **PMMA cements** present many drawbacks, such as their potential shrinkage, their highly exothermic polymerization (between 67 and 124 °C) which can lead to thermal necrosis of the tissues, a possible formation of a fibrous

capsule around the cement because no chemical bond with bone was established (bioinertia), the toxicity of released unreacted methyl methacrylate (MMA) (Lewis, 1997), and the deterioration of cement–implant or cement–bone interface with time, leading to instability and problems of mechanical failure.

Other kinds of cements have been investigated in the literature. Many consist in an improvement of classical cements with various loads such as drugs (Kaplan et al., 2012), ions (Thormann et al., 2013) and bioactive components such as bioglass (Yu et al., 2013). However, even though it has been decades since the first use of bone cements, no innovative cement has been used clinically and, as far as we know, improvements which have been made still remain uncommon and not groundbreaking.

Amongst those trials, a minority use hydrogels. Hydrogels are polymers which have the ability to absorb water. One can find them in the composition of contact lenses for example. About four decades ago, in order to counter the drawbacks of commercial acrylic cements, Greenberg and Kamel (Greenberg et al., 1976) began the investigation on hydrogel-based bone cements, developing a cement for dental applications using acrylic acid (AA), an acrylic hydrogel, and alumina. The obtained cement was biocompatible, easy to process, had good mechanical properties, interconnected porosity and was able to swell. Later on, Boesel et al. continued this study using AA first (Boesel et al., 2004) and then 2-hydroxyethyl methacrylate (HEMA) (Boesel and Reis, 2004) as a liquid base to mix with a powder blend composed of starch, cellulose acetate and a bioactive ceramic load. HEMA is another acrylic monomer which gives, in its polymerized form, a hydrogel used in many biological and biomedical applications such as contraceptive intravaginal rings (Han et al., 2009), embedding medium for tissues under optical microscopy, drug delivery vectors, cell culture, vascular implants, and contact lenses (Montheard et al., 1992). This kind of cement used in Boesel et al.'s study (Espigares et al., 2002) was supposed to enable the swelling of the cement *in vivo* to get bone cavities completely filled, as well as enhanced degradability and bioactivity. More recently, HEMA was also used in an α -TCP/HEMA bone cement by Hurle et al. (Hurle et al., 2016). To the best of our knowledge, few cements use a combination of HEMA, cellulose acetate, starch and a bioceramic filler, and moreover the formulation of that kind of cement was empirically determined by a trial and error approach. The chosen bioceramic is alumina for its excellent biocompatibility, but other ceramics as hydroxyapatite, α or β - tricalcium phosphate or bioglasses are also candidates of choice for their high bioactivity.

In the orthopedic field, biodegradable injectable materials have attracting interest in bone regeneration. Ideally, the synthetic bone cements should be highly biocompatible, promote tissue formation, as well as be degradable in response to the formation of a new bone.

They should have appropriate mechanical and rheological properties, adhere and integrate with the surrounding native tissue and properly fill the injured site, thus providing an important step towards minimally invasive orthopedic procedures.

In addition, in the formulation used, HEMA hydrogel allows the cement to rehydrate on contact with fluids. This swelling can be interesting to compensate for the shrinkage that occurs after setting. Therefore, these 3 parameters have been studied: 1/ the compressive Young's modulus E (in MPa), 2/ the swelling rate and 3/ the complex shear modulus G^* (in Pa) which is related to rheological properties of the cement.

In this study, a mixture design is used to optimize the formulation of the powder phase of the cement regarding the proportions of cellulose acetate, starch and bioceramic in order to achieve the required properties of HEMA-based bone cements in terms of rheological properties, compressive strength and swelling rate. Mixture design is a special topic within statistical Design of Experiments (DoE) which is a well-established, efficient and time-saving tool which focuses on how to plan and conduct experiments in order to produce the maximum amount of relevant information from the collected data with a minimum number of well-chosen experimental runs (Ericksson et al., 2008).

The objective was to obtain a HEMA-based bone cement **with hydrogel properties**, which does not shrink for bone filling, with optimized rheological and mechanical properties by adapting the powder content of the cement. The strength of this work is to offer a powerful method capable of adapting the formulation of the cement according to desired characteristics.

2. MATERIALS AND METHODS

2.1. Cement composition and preparation

All materials were purchased from Acros Organics, USA, unless otherwise mentioned, and were used as received.

Composite starch-based acrylic bone cements were investigated. They were composed of two phases:

- One solid phase containing the bioceramic filler: alumina (Al_2O_3 , Almatis GmbH, Germany), corn starch (Unilever France, France) and cellulose acetate powders, with the addition of benzoyl peroxide (BPO), initiator of the polymerization;
- One liquid phase containing HEMA (stabilised, 97%) the acrylic monomer, with the addition of N,N-dimethyl toluidine (DMT), activator of the polymerization (Boesel et al., 2004).

The total weight was kept fixed at 4 g for all prepared samples with a constant solid-to-liquid ratio (S/L) of 60/40 in weight, which ensured a workable consistency of the cement pastes.

Both phases were thoroughly mixed together during 30 s by hand with a metal spatulain silicone rubber moulds. The polymerization of HEMA and thus the hardening of the cement were achieved by the activation of BPO by DMT and the subsequent release of free radicals during that mixing phase.

Then, the mixtures were either used without further treatment for rheological tests, or put into cylindrical silicone rubber moulds with a diameter of 12 mm and a height of 20 mm, and let to cure for swelling and mechanical tests.

2.2. Powder characterization

Powders particle size distribution and especially the median particle size (D50) were determined using dry phase laser diffraction granulometry according to Fraunhofer optical model with an LS particle size analyzer (Beckman Coulter, CA, USA). Specific surface area of the powders particles was determined by N_2 absorption according to the Brunauer-Emmett-Teller (BET) method (Autopore IV 9505, Micromeritics, GA, USA). Density of the powder particles was measured by helium pycnometry (AccuPyc 1330, Micromeritics, GA, USA). Alumina powder was investigated by X-ray diffraction (XRD) (D2 Phaserdiffractometer, Bruker Co, France) using $\text{CuK}\alpha$ radiation ($\lambda=0.1542$ nm) in the 2θ range 6-60° to check the sample purity and the crystallization state.

2.3. Mixture Design

2.3.1 Definition of mixture factors and responses

An efficient way of investigating a blending process in which all ingredients sum to 100% is to use the mixture design approach for changing composition and exploring how such changes

will affect the properties of the mixture (Ericksson et al., 1998; Ericksson et al., 2008; Cornell et al., 2002). Many of the basic DoE concepts are applicable for mixture design, especially the modelling one which consists in connecting the results by means of a mathematical model. The model is then used for interpretation, prediction and optimization (see section 3.2).

In this study, the mixture design concerns exclusively the solid phase content of the cement and the three components (mixture factors) are the proportions in weight of the bioceramic (X_1 – alumina) and of the two polysaccharides (X_2 –corn starch and X_3 –cellulose acetate).

For each mixture composition verifying the overall external constraint $X_1 + X_2 + X_3 = 1$, three variables of interest (responses) were measured:

- the compressive Young's modulus E (in MPa), referred to as response Y_E , which is related to compressive mechanical properties;
- the swelling rate (response Y_S in %);
- the complex shear modulus G^* (in Pa) which is related to rheological properties of the material (response Y_G in Pa).

Sections 2.4 to 2.6 describe in details how the three responses are determined experimentally.

The experimental objective of this study was optimization for finding a mixture composition that 1) maximizes the Young's modulus Y_E , 2) minimizes the swelling rate Y_S and 3) gives a complex shear modulus Y_G in the range 1500 - 40000 Pa (Farrar et al., 2001; Ly et al., 2017). In addition to the criteria required for each response, the optimal mixture formulation should include a significant amount of bioceramic which was considered as relevant in the range $X_1 = [0.3, 0.75]$.

2.3.2 Mixture model, mathematical and statistical analysis

The specific DoE software MODDE 8.0 (Umetrics, Umea, Sweden) was used to build the design, and then to carry out mathematical, graphical and statistical analysis of the collected data.

Let us remind that the modelling concept is of major importance in the experimental strategy; as optimization was the objective, each response was modelled with the quadratic polynomial Cox regression model (Wold et al., 1984) given by equation (1):

$$\hat{Y} = \beta_0 + \sum_{i=1}^q \beta_i X_i + \sum_{i=1}^q \beta_{ii} X_i^2 + \sum_{i=1}^q \sum_{i < j}^q \beta_{ij} X_i X_j \quad (1)$$

Where X_i is the amount of each powder and β 's are the model coefficients : β_i , β_{ii} and β_{ij} ($i \neq j$) are factor linear effect, factor quadratic effect and two-factor interaction respectively.

For a three-component mixture ($q = 3$), this model includes $p = 10$ meaningful and easy to interpret coefficients which are assessed using the partial least squares (PLS) regression method (Kettaneh-Wold., 1992; Cox, 1971).

Once the coefficients are assessed, the model must be statistically evaluated prior to being used for prediction and optimization. Many diagnostic tools for model evaluation are proposed by MODDE and three of them were used in this study: 1) the most well-known goodness of fit R^2 and goodness of prediction Q^2 statistics which should be as close to unity as possible, and preferably not be separated by more than 0.2-0.3 (Ericksson et al., 2008), 2) the analysis of variance (ANOVA) (Box et al., 2005) and 3) the evaluation of the model residuals. The last two tools will be described and discussed in Supplementary Information (SI).

2.3.3 Definition of Experimental domain and generation of the mixture design

For a three-component mixture, the experimental domain is a regular two-dimensional simplex (Figure 1a) when all proportions can vary from 0 to 1. In our study, lower and upper bounds, gathered in Table 1, were selected on the basis of the results of three preliminary experiments (points No. 1 to 3 in Table 2) yielding an irregular experimental domain as shown in Figure 1.

Table 1: lower and upper bounds of the experimental domain

Mixture factor	X_1 - Alumina	X_2 - Corn starch	X_3 - Cellulose acetate
Lower bound	0.3	0.1	0.1
Upper bound	0.75	0.5	0.4

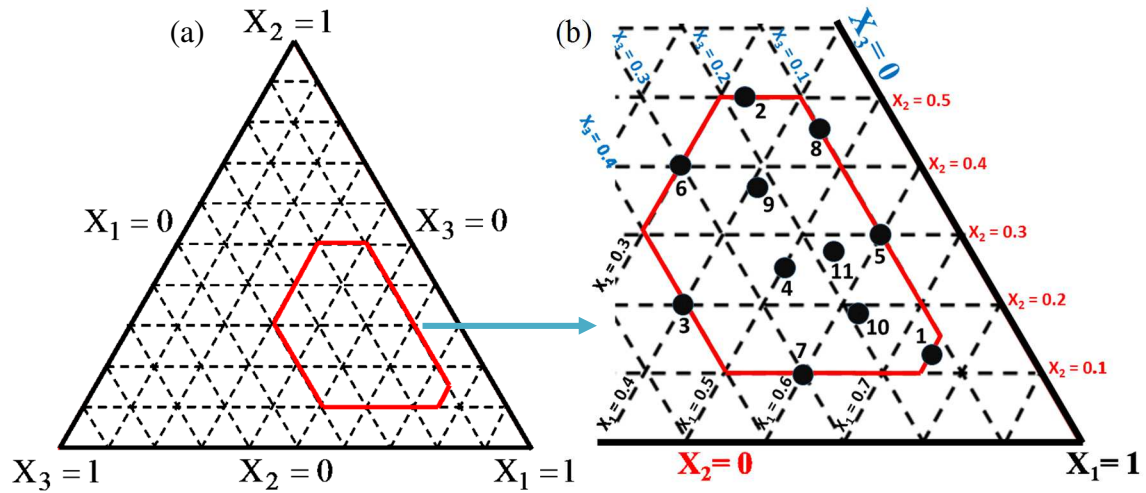


Figure 1: (a) studied experimental domain delimited with red lines, (b) distribution of the chosen points located inside and at the boundaries of the domain.

An irregular experimental region cannot be addressed by any classical mixture design. Consequently, a D-optimal design was computer generated with MODDE. The selected design, characterized by a satisfactory G-efficiency value of 74%, includes 8 points labelled from 1 to 8 in Table 2. Most of the points are located at the boundaries of the domain, ensuring a homogenous coverage of the domain. For optimization purpose, it is relevant that a few mixtures within the experimental region are available. Consequently, two useful extra points (No. 9 and 10) were added to the design. The fourth preliminary test run (point No. 11), even though its position is close enough to points No. 4 and 5, was also included. Eventually, the design consisted in 11 points labelled from 1 to 11 shown in Figure 1b with their compositions in Table 2.

To assess the experimental reproducibility, experiments No. 4 to 8 which are well spread throughout the experimental domain were measured three times. Such replicated runs will enable the replicate (pure) error to be evaluated as well as statistical tests such as the Lack-Of-Fit test to be achieved (see SI). As points No. 1, 2, 3, 9, 10, 11 were measured only once, the final design includes a total of 21 runs.

Table 2: composition of the 11 chosen points in the experimental domain (Figure 1).

Exp. No.	X1 - alumina	X2 - corn starch	X3 - cellulose acetate
1	0.75	0.125	0.125
2	0.3333	0.5	0.1667
3	0.4	0.2	0.4
4	0.5	0.25	0.25
5	0.6	0.3	0.1
6	0.3	0.4	0.3
7	0.6	0.1	0.3
8	0.45	0.45	0.1
9	0.41	0.37	0.22
10	0.63	0.185	0.185
11	0.55	0.27	0.18

2.4. Mechanical test

After curing, the cylindrical cement samples were gently stirred in 50 ml of phosphate buffered saline (PBS) at ambient temperature, for seven days.

Compressive mechanical tests were conducted on a universal testing machine (AGS-X, Shimadzu, Japan) with a 10-kN load cell. They consisted of a pre-test which enabled the crosshead to go down at a speed of 2 mm/min until it touched the upper endplate of the sample, after which the genuine compression test began with a crosshead speed of 20 mm/min till 60% of the sample's deformation in height (Boesel et al., 2004). The compressive Young's modulus (E) was measured; it constitutes one of the three responses used in the mixture design: Y_E .

2.5. Swelling test

The cement in contact with PBS hydrated and increased in volume and weight as a function of the duration of immersion. After immersion, the samples were weighed dry right after curing and before immersion (day 0), and weighed wet at 7 days of immersion. For the wet weight, samples were picked out of water and excess water was removed by dabbing the sample with a humid piece of absorbent paper before being weighted. Swelling rate S (in %) was defined as the ratio of the difference between the weight after immersion (W_{wet}) and the weight before immersion (W_{dry}) to the weight before immersion, multiplied by 100 (Equation 3).

$$S (\%) = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100 \quad (3)$$

This value was taken as the second variable of interest in the mixture design: Y_s .

2.6. Rheology

Rheology of the cement pastes was tested by the means of a table-top rotational rheometer (MCR 102, Anton Paar, Austria). A parallel-plate configuration was used, with a diameter of plates of 25 mm and a gap between plates of 1.5 mm. While the gap should be significantly greater (at least five to ten times; Van Wazer et al., 1963) than the size of the biggest particle of the mixture, this gap was chosen in order to avoid liquid-like paste leakage.

BPO and DMT, which make the cement paste cure, were not used in order to allow us to use the rheometer without any risk of damage on the measurement device.

The solid and liquid phases were mixed as described in section 2.1. for 30 s until a homogenous paste was obtained, before being loaded onto the bottom static plate of the geometry with a spatula. The top plate was then put to contact with the sample until the gap was reached, excess material was removed by trimming and the measurement in dynamic oscillation mode of the complex shear modulus $|G^*|$ began 2 min after the beginning of the mixing with an oscillatory shear of 1% at a frequency of 1 Hz. The viscoelastic properties of bone cements are a function of time. The complex shear modulus $|G^*|$ was recorded at room temperature, 7 min after the beginning of the mixing to be close to per-operative conditions. This is the third response Y_G considered in the mixture design.

Briefly, the complex shear modulus $|G^*|$ represents the material's resistance to strain and is a characteristic of viscoelastic materials. It is obtained by combination of the storage modulus (or elastic modulus) G' which describes the solid-like behaviour of the material, and the loss modulus (or viscous modulus) G'' which describes the liquid-like modulus of the material, according to the following equations 4 and 5:

$$|G^*| = \sqrt{G'^2 + G''^2} \quad (4) \quad \text{and} \quad G^* = G' + i G'' \quad (5)$$

Where i is the imaginary unit. Details about the complex shear modulus, shear storage and shear loss moduli can be found elsewhere (Farrar et al., 2001; Aho et al., 2015).

3 Results

3.1 Physico-chemical characteristics

The different physico-chemical characteristics (powder D₅₀ particle size, BET surface area and apparent density) are presented in Table 3.

Table 3: powder characterizations: granulometry, surface area and apparent density.

	D ₅₀ particle size (μm)	BET surface area (m ² /g)	Density
Alumina	1.09	1.64 ± 0.02	3.96
Corn starch	13.97	0.55 ± 0.02	1.52
Cellulose acetate	218.00	6.73 ± 0.02	1.37

The powder particle size distribution (Figure 2) showed very different sizes for each powder: alumina and corn starch are significantly smaller than cellulose acetate.

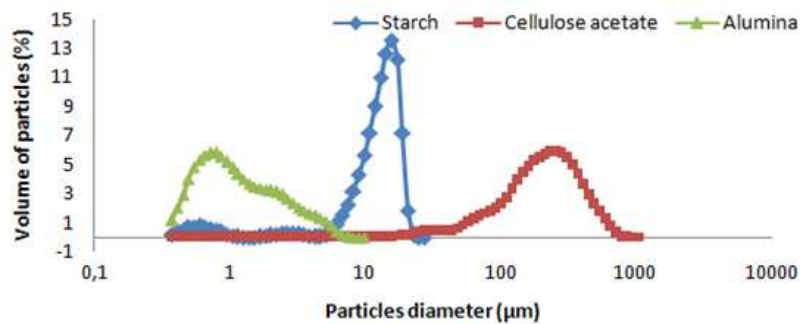


Figure 2: particle size distribution of alumina, corn starch and cellulose acetate.

The DRX analysis of bioceramic powder (Figure 3) can be perfectly indexed as pure alumina (no impurity phase). The very sharp peaks indicate a high crystallinity of the powder.

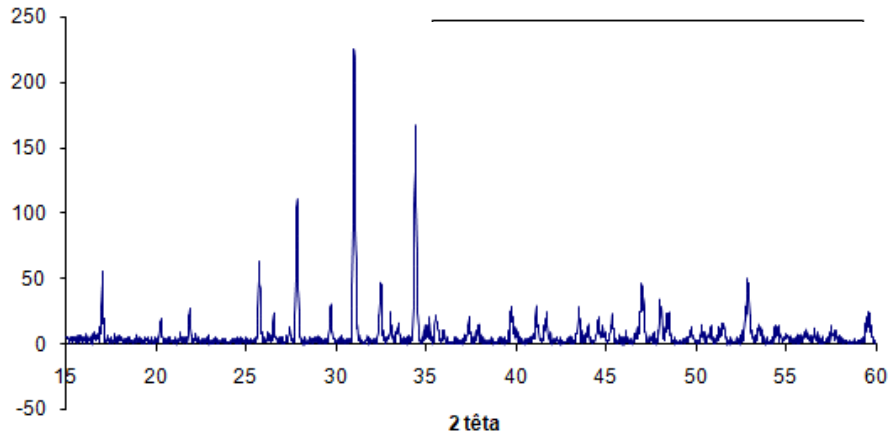


Figure 3: XRD pattern of pure alumina.

3.2 Mixture design

3.2.1 Raw data analysis

The response values of the 21 runs, listed in Table 4, enable to define more accurately acceptable thresholds associated with each response:

1– The Young's modulus Y_E should be larger than 5 MPa; this value remains lower than that of the trabecular bone which range between 50 and 500 MPa (Eliaz et al., 2017) but compatible with hydrogel formulations (HEMA derived) where Young's modulus is lower (0.2 to 0.5 MPa) (Chung et al., 2005).

2 - The cement formulation contains hydrogel because a swelling effect is desired to best fill the bone defect. Excessive swelling is not necessary as it may be deleterious. That is why an arbitrary limit was chosen and set at 24.5%. Therefore, the swelling rate Y_S , which does not exceed 24.5%, sought that the *in situ* cement is in contact with the bone tissue, but with moderate swelling so as not to damage the recipient site.

3–The complex shear modulus Y_G must be low enough to enable easy injectability via a syringe and catheter system but not too weak to avoid all complications related to leakage. This modulus should be between:

- either 2000 to 4000 Pa to maintain a low viscosity for use with a cement gun
- or 10000 and 40000 Pa corresponding to a medium viscosity for use as paste, according to the literature (Farrar et al., 2001; Ly et al., 2017).

Table 4: formulations and results of 21 runs included in the mixture design.

Exp. No.	X1 - alumina	X2 - corn starch	X3 - cellulose acetate	YE (MPa)	YS (%)	YG (Pa)
1	0.75	0.125	0.125	1.5	22.9	141
2	0.3333	0.5	0.1667	2.9	28.2	1526
3	0.4	0.2	0.4	31.3	27.5	154660
4	0.5	0.25	0.25	8,0	25.7	10434
5	0.6	0.3	0.1	1.5	26.3	64
6	0.3	0.4	0.3	9.8	28.1	73381
7	0.6	0.1	0.3	9.5	24.9	26087
8	0.45	0.45	0.1	0.6	31.4	89
9	0.41	0.37	0.22	4.1	28,0	7022
10	0.63	0.185	0.185	4.1	25,0	649
11	0.55	0.27	0.18	3.6	25.4	2042
12	0.5	0.25	0.25	8,0	25.5	4920
13	0.6	0.3	0.1	1.9	26.2	25
14	0.3	0.4	0.3	6.2	28.1	96704
15	0.6	0.1	0.3	8.9	24.7	31260
16	0.45	0.45	0.1	0.6	31.3	129
17	0.5	0.25	0.25	9.1	25.6	7549
18	0.6	0.3	0.1	1.7	26.2	71
19	0.3	0.4	0.3	7.3	28,0	103910
20	0.6	0.1	0.3	8,0	24.8	29766
21	0.45	0.45	0.1	0.6	30.8	241

Cells in Table 4 were highlighted in green when the criterion is fulfilled. It is worth noting that no formulation fulfils the three criteria simultaneously. However, mixture No. 7 and its replicates No. 15 and 20 with proportions (X_1 , X_2 , $X_3 = 0.6$, 0.1 and 0.3) show satisfactory values for responses Y_E and Y_G , and almost acceptable values for the swelling rate (around 24.8% which is slightly higher than the threshold fixed at 24.5%). It can reasonably be thought that optimal mixtures will be close to this formulation.

Prior to performing mathematical analysis with PLS regression, raw data analysis was carried out which showed that a response transform using the Box-Cox method (Box et al., 1964) had to be applied to both Y_E and Y_G responses (see SI). A response transform has a twofold impact: i) ensuring a distribution of responses as close to normality as possible, which is recommended in regression analysis (Montgomery., 2001) and ii) making transformed values of the same order of magnitude, otherwise either replicate (pure) error evaluation or Lack-of-fit test cannot be carried out properly. The logarithmic function $Z = \log (C*Y)$, which is one of the functions implemented in MODDE, was shown to be the most appropriate response transform to apply with the constant C taken at 10 and 1 respectively for Y_E and Y_G responses (see SI).

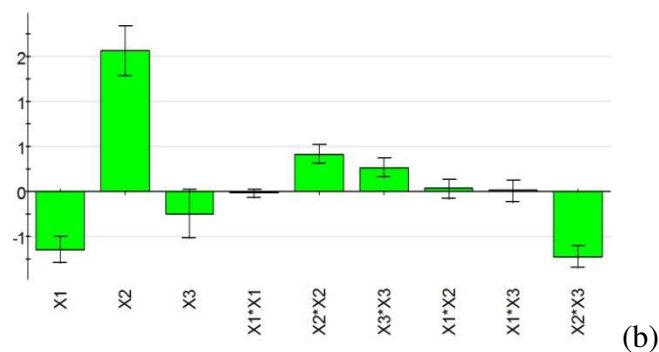
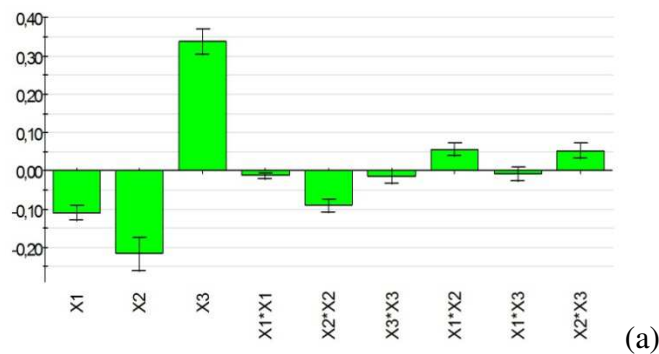
3.2.2 Modelling results

Let us remind that each response Z_E , Y_S and Z_G was modelled with the quadratic polynomial Cox regression model (Wold et al., 1984) given by equation (1). After using and interpreting diagnostic tools for model evaluation provided by MODDE, the final PLS regression analysis of Z_E , Y_S and Z_G response values gave models with R^2 and Q^2 determination coefficients gathered in Table 5. These statistics strongly point in direction of valid quadratic models which will be used now for interpretation, prediction and optimization.

Table 5: Goodness of fit R^2 and goodness of prediction Q^2 for $Z_E = \log(10.Y_E)$, Y_S and $Z_G = \log(Y_G)$ responses.

Response	$Z_E = \log(10.Y_E)$	Y_S	$Z_G = \log(Y_G)$
R^2	0.991	0.985	0.986
Q^2	0.965	0.895	0.911

Regression coefficients of the computed quadratic models are displayed in Figures 4 for responses $Z_E = \log(10.Y_E)$ (a), Y_S (b) and $Z_G = \log(Y_G)$ (c).



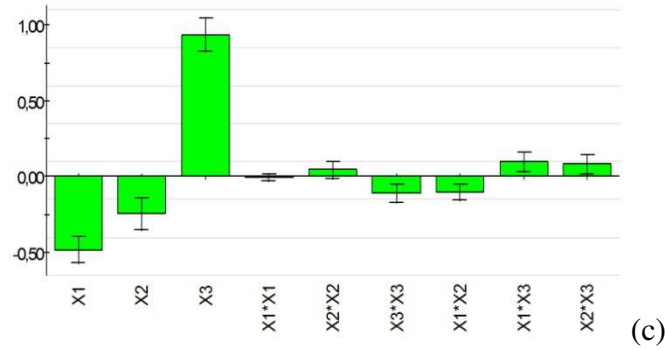


Figure 4: Coefficient plots for responses $Z_E = \log(10 \cdot Y_E)$ (a), Y_S (b) and $Z_G = \log(Y_G)$ (c). For each coefficient, the vertical bar corresponds to the computed 95% confidence interval.

Coefficient plots in Figures 4a and 4c show that the amount of cellulose acetate (X_3) has a major positive effect on both log-transformed responses Z_E and Z_G . Thus these response values, and consequently Y_E and Y_G (when is applied the inverse response transform $Y_E = 10^{Z_E - 1}$ and $Y_G = 10^{Z_G}$ respectively), are both maximized with high cellulose acetate proportion. In other terms, both mechanical and rheological properties are enhanced with an increase of the amount of cellulose acetate in the blend.

The improvement of mechanical properties with higher amount of cellulose acetate does not appear surprising as cellulose acetate as well as other cellulose esters are used for the benefits they bring to other polymers concerning mechanical strength (Brangança et al., 2001; Edgar et al., 2001). In addition, there is probably a compatibility of cellulose acetate with the other polymers that would prevent the mechanical properties of the samples from decreasing when its amount is increased.

The major effect of cellulose acetate on rheological properties was underlined in Appaw et al.'s work (Appaw et al., 2007) which studied the effect of the concentration of cellulose acetate in a mixed solvent system composed of cellulose acetate, N,N-dimethyl acetamide and water. The authors noted that an increase of the amount of cellulose acetate in the system led to an increase of the viscosity of the solution and consequently of both G' and G'' and thus an increase of G^* .

Furthermore, it is much likely that the more cellulose acetate, the larger the G^* value because of the entanglement of the polymeric chains.

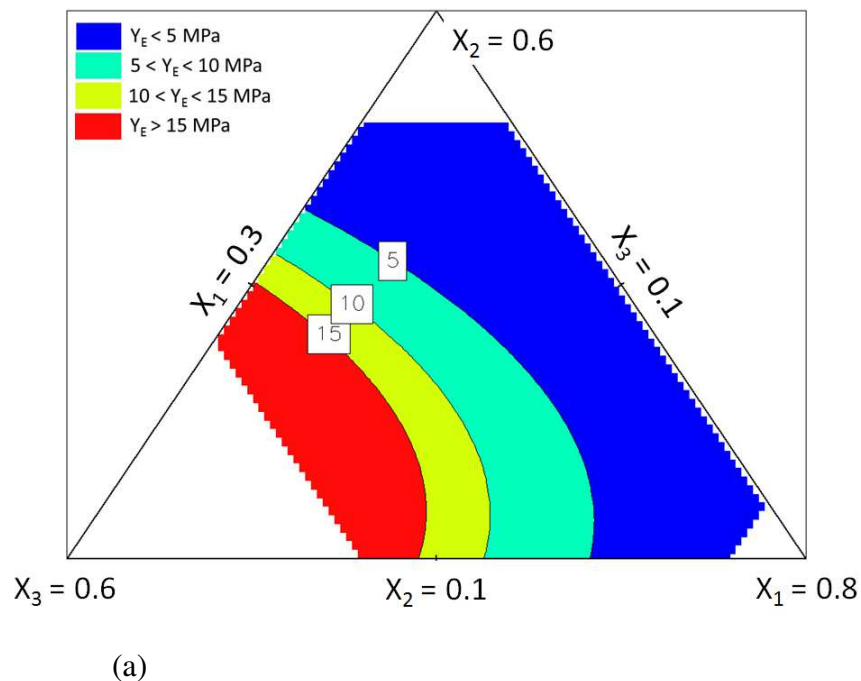
In addition, the hydroxyl groups of cellulose acetate may bind to the carbonyl groups of HEMA. Consequently, when the amount of cellulose acetate increases, more hydrogen

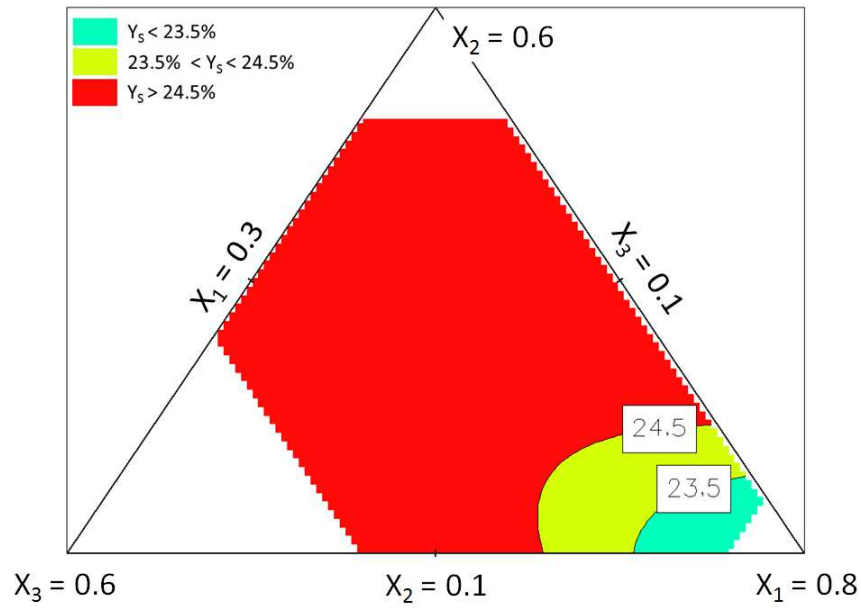
bounds may be created as a result of this interaction, hence the higher rheological properties of the system (Appaw et al., 2007; Morita, 2014).

As shown in Figure 4b, the proportion of corn starch X_2 has a strong positive effect on swelling properties and the swelling rate Y_S is maximized with high X_2 amount. The same feature was already observed by Pereira et al. (Pereira et al., 1998). The tendency of starch to increase the swelling of the material might be explained by its structure. Indeed, starch is a natural polysaccharide composed of amylose and amylopectin, two homopolymers of glucose, to which water can bind. The high proportion of amylopectin in starch and the presence of numerous hydroxyl groups, to which water particularly binds, are potential reasons for the increased swelling in presence of starch. In our case, a moderate swelling rate is requested ($< 24.5\%$) which can be obtained for low X_2 amounts.

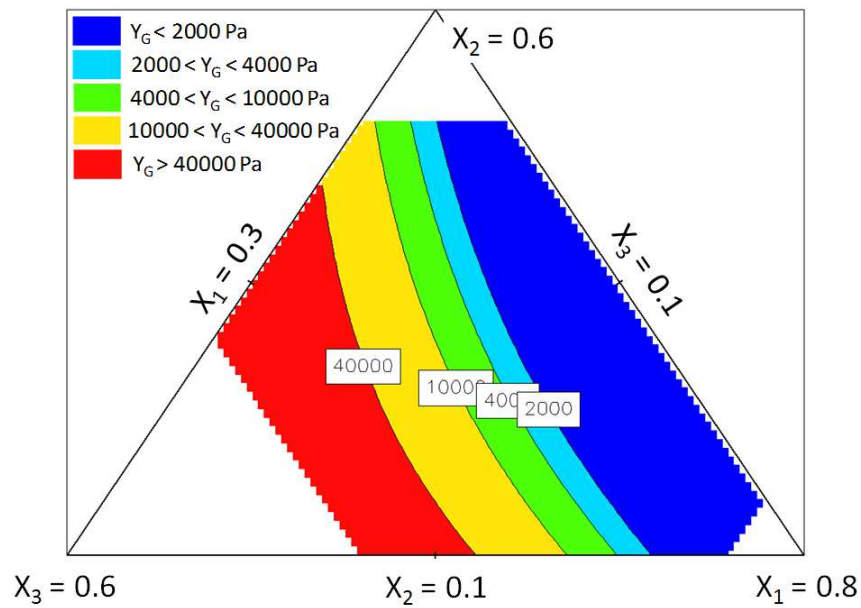
3.2.3 Using models for prediction and optimization of the formulation

Coefficient plots in Figure 4 also show the presence of significant (at 95% confidence level) square and/or interaction terms, more particularly for Z_E and Y_S responses. These effects can be more easily understood by means of mixture contour plots displayed in Figure 5 where inverse response transforms for Z_E and Z_G are computed by MODDE so that real values are given for Y_E (in MPa) and Y_G (in Pa).





(b)



(c)

Figure 5: Mixture contour plots for the 3 responses: Young's modulus Y_E (in MPa) (a), Swelling rate Y_S (in %) (b) and Complex shear modulus Y_G (in Pa) (c)

Acceptable minimum (resp. maximum) thresholds of 5 MPa (resp. 24.5%), defined in section 3.2.1, are indicated in Figure 5a (resp. 5b) for Young's modulus Y_E (resp. Swelling rate Y_S) responses. Acceptable variation ranges [2000-4000] or [10000-40000] MPa for the rheological response Y_G are shown in Figure 5c. Whereas the criterion $Y_E > 5$ MPa can be

fulfilled in a still large part of the experimental domain, acceptable values for the two other responses are reachable in a significantly reduced part of the domain.

The use of separate contour plots does not enable to predict easily whether an optimal experimental region can satisfy the three criteria simultaneously. An overlay of relevant parts of the three contour plots is shown in Figure 6 which demonstrates that optimal regions highlighted in green are reduced to a very small part of the initial experimental domain.

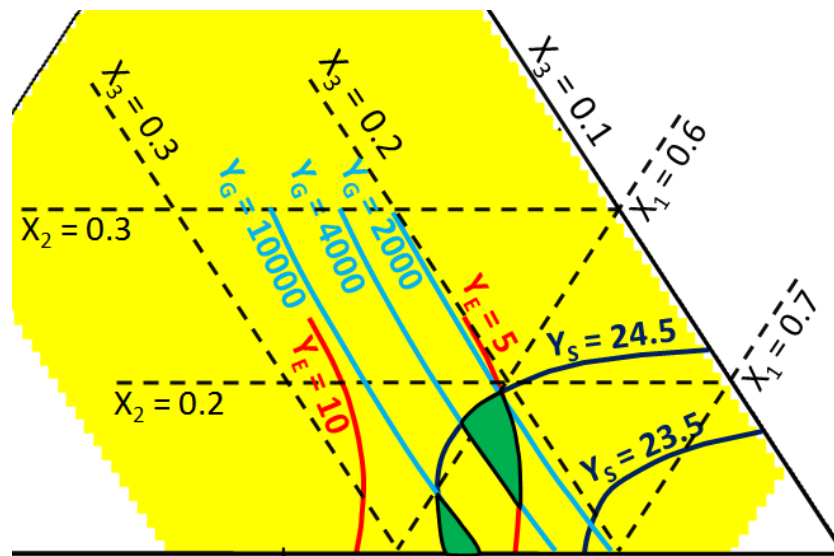


Figure 6: Overlay of contour plots showing two predicted regions of interest (in green)

With such very small regions of interest, the choice of optimal formulations is rather limited. By using the MODDE optimizer functionality, a good compromise between the criteria associated with all responses was obtained with optimal mixture points defined by the proportions either $[X_1, X_2, X_3 = 0.615, 0.165, 0.220]$ or $[X_1, X_2, X_3 = 0.63, 0.11, 0.26]$, depending on the position of the predicted region (in green in Figure 6). As expected (see section 3.2.1), this latter combination is very close to that of mixture No. 7 shown in Table 4.

Model predictions together with 95% confidence intervals (lower and upper limits) computed by the software for the three responses are summarized in Tables 6 and 7 for both optimal formulations.

Table 6: Model predictions of Y_E , Y_S and Y_G responses for the optimal formulation [X_1 , X_2 , $X_3 = 0.615, 0.165, 0.220$].

Response	Predicted value	95% Confidence interval	
		Lower	Upper
Y_E (MPa)	5.5	4.9	6.0
Y_S (%)	24.2	23.9	24.5
Y_G (Pa)	3141	2223	4437

Table 7: Model predictions and measurement results of Y_E , Y_S and Y_G responses for the optimal formulation [X_1 , X_2 , $X_3 = 0.63, 0.11, 0.26$]. Standard deviation is indicated in parentheses for the measured mean value.

Response	Predicted value	95% Confidence interval		Measured mean value (3 repetitions)
		Lower	Upper	
Y_E (MPa)	6.2	5.4	7.0	6.8 (2.2)
Y_S (%)	24.3	23.9	24.7	24.3 (0.1)
Y_G (Pa)	10,448	6743	16189	12515 (1320)

In Table 7, it is worthwhile noting that the lower to upper limits of the confidence interval for Y_E and Y_G responses cover a rather large response range. By using the models in Z-values (equation 1) and keeping the proportion X_2 at its optimal value 0.11, this range corresponds for both responses to a variation of X_3 (cellulose acetate proportion) from around 0.245 to 0.275. So, we can see that rather small variations of X_3 can cause very fast changes of predicted Y_E and Y_G values due to both the strong effect of X_3 amount on Z-predicted values (Figures 5a and 5c) and the mathematical form of inverse response transforms $Y_E = 10^{Z_E - 1}$ and $Y_G = 10^{Z_G}$. In the validation step, small final adjustments of the mixture factors are often required to fulfil the criteria associated with all responses simultaneously.

Three verifying experiments were carried out for the optimal formulation showing the highest amount of bioceramic X_1 (0.63). The results reported in Table 7 indicate that the models predict well since the measured mean value for each response is included in the confidence

interval. Consequently the formulation of the optimal mixture is validated and no final adjustment is needed in the present study.

These features led us to optimize the amounts of each component to obtain a cement with adequate properties, while maximizing the presence of bioceramic. Alumina was chosen here for its excellent biocompatibility, but other ceramics such as hydroxyapatite, α or β tricalcium phosphate, hemihydrated calcium sulphate, or bioglasses are also candidates of choice for their high bioactivity.

4. Conclusion

The use of the mixture design is a relevant approach in the field of development and validation of a new bone cement formulation. When a mixture design is implemented, relevant issues to be considered are selection of the most convenient proportion of each component, selection of the most appropriate regression mathematical model depending on the expected information, interpretation and understanding of the influence of mixture components on output variables of interest (responses) and determination of the optimal formulation depending on the desired characteristics of the cement.

In this study, a computer-generated mixture design was used to investigate how the amount of bioceramic filler, cellulose acetate and corn starch incorporated in an original HEMA-based bone cement could affect its rheological, mechanical and swelling properties characterized by the complex shear modulus, compressive modulus and swelling rate respectively. Each response was modelled with a polynomial quadratic regression model, which was evaluated and validated (at 95% confidence level) using several diagnostic tools.

Modelling results showed that the rheology of the cement as well as its mechanical strength were mainly impacted by the proportion of cellulose acetate, whereas its swelling was mainly modulated by the presence of starch. The overlay of contour plots demonstrated that the region of interest where the desired properties of the cement are optimal is located in a very small part of the initial experimental domain.

The results obtained for verifying experiments with the optimal formulation are in good agreement with model predictions together with 95% confidence intervals. Final use of models enabled optimization of the formulation of HEMA-based bone cement in order to achieve the different required properties.

By limiting the number of tests and experiments, this simple and attractive method can be applied for other desired properties, ie the mechanical properties and especially the rheological behavior adapted to the desired clinical application.

References

Aho, J., Boetker, J.P., Baldursdottir, S., Rantanen, J. 2015. Rheology as a tool for evaluation of melt processability of innovative dosage forms. *Int J Pharmaceut.* 494, 623–642.

Appaw, C., Gilbert, R.D., Khan, S.A., 2007. Viscoelastic behavior of cellulose acetate in a mixed solvent system. *Biomacromolecules.* 8, 1541–1547.

~~Blue, D., Nguyen, N.D., Alarkawi, D., Nguyen, T.V., Eisman, J.A., Center, J.R., 2015. Accelerated bone loss and increased post fracture mortality in elderly women and men. *Osteoporosis Int.*; 26 (4), 1331–1339.~~

Boesel, L.F., Mano, JF, Reis, R.L., 2004. Optimization of the formulation and mechanical properties of starch based partially degradable bone cements. *J. MaterSci: Mater M.* 15, 73–83.

Boesel, L.F., Reis, R.L., 2004. Hydrophilic matrices to be used as bioactive and degradable bone cements. *J. Mater.Sci.: Mater M.* 15, 503–506.

Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. *J R Stat Soc.* 26, 211–252.

Box, G.E.P., Hunter, W.G., Hunter, J.S., 2005. *Statistics for experimenters: design, innovation and discovery.* 2nd ed. New York: John Wiley & Sons.

Bragança, F.C., Rosa, D.S., 2003. Thermal, mechanical and morphological analysis of poly(ϵ -caprolactone), cellulose acetate and their blends. *PolymAdvan Technol.* 14,669–675.

Carvalho P.P. Rodrigues M.T. Reis R.L. Gomes M.E., 2016, Starch-Based Blends in Tissue Engineering, Biomaterials from Nature for Advanced Devices and Therapies, First Edition, Chapter 15, 14 October 2016 <https://doi.org/10.1002/9781119126218.ch15>

Charnley, J., 1960. Anchorage of the femoral head prosthesis to the shaft of the femur. *J Bone Joint Surg.* 42B, 28–30.

Chung, J.T., Vlugt-Wensink, K.D.F., Hennink, W.E., Zhang, Z., 2005. Effect of polymerization conditions on the network properties of dex-HEMA microspheres and macrohydrogels. *Int J Pharmaceut.* 288, 51–61.

Cornell, J.A., 2002. Experiments with mixture-designs, models, and the analysis of mixture data. 3rd ed. New York, John Wiley & Sons.

Cox, D.R., 1971. A note on polynomial response functions for mixtures. *Biometrika.* 58, 155–159.

Edgar, K.J., Buchanan, C.M., Debenham, J.S., et al., 2001. Advances in cellulose ester performance and application. *Prog Polym Sci.* 26, 1605–1688.

Eliaz, N., Metoki, N., 2017. Calcium phosphate bioceramics: A review of their history, structure, properties, coating technologies and biomedical applications. *Materials.* 10, 334.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wikstroem, C., Wold, S., 2008. Design of experiments: Principles and applications. 3rd ed. Umea: Umetrics.

Eriksson, L., Johansson, E., Wikstroem C., 1998. Mixture design: Design generation, PLS analysis and model usage. *Chemo metr Intell Lab Syst.* 43, 1–24.

- Espigares, I., Elvira, C., Mano, J.F., Vázquez, B., San Román, J., Reis, R.L., 2002. New partially degradable and bioactive acrylic bone cements based on starch blends and ceramic fillers. *Biomaterials*. 23, 1883–1895.
- Farrar, D.F., Rose, J., 2001. Rheological properties of PMMA bone cements during curing. *Biomaterials*. 22, 3005–3013.
- Ginebra, M.P., Canal, C., Espanol, M., Pastorino, D., Montufar, E.B., 2012. Calcium phosphate cements as drug delivery materials. *Adv Drug Deliver Rev*. 64, 1090–1110.
- Greenberg, A.R., Kamel, I., 1976. Polymer-ceramic composite for tooth-root implant. *J Biomed Mater Res*. 10, 777–788.
- Han, Y. A., Lee, E. M., Ji, B.C., 2009. The physical properties of poly(2-hydroxyethyl methacrylate) copolymer hydrogels used as intravaginal rings. *Chinese J Polym Sci*. 27, 359–366.
- Hurle, K., Christel, T., Gbureck, U., Moseke, C., Neubauer, J., Goetz-Neunhoeffler F., 2016. Reaction kinetics of dual setting α -tricalcium phosphate cements. *J Mater Sci: Mater Med*. 27, 1.
- Kaplan, L., Kurdziel, M., Baker, K.C., Verner, J., 2012. Characterization of daptomycin-loaded antibiotic cement. *Orthopaedics*. 35, e503–e509.
- Kettaneh-Wold, N., 1992. Analysis of mixture data with partial least squares. *Chemometr Intell Lab Syst*. 14, 57–69.
- Lewis, G., 1997. Properties of acrylic bone cement: State of the art review. *J Biomed Mater Res (Appl Biomater)*. 38, 155–182.

Liu M., Zeng X., Ma C., Yi H., Ali Z., Mou X., Li S., Deng Y. and He N., 2017, Injectable hydrogels for cartilage and bone tissue Engineering, *Bone Research* 5, 17014.

Ly, O., Monchau, F., Rémond, S., et al., 2017. Formulating and optimizing the rheological properties of a composite bone cement using mixture design. *Materials, Methods & Technologies*. 11, 403–419.

Montgomery, D.C., 2001. Response surface methods and other approaches to process optimization. *Design and analysis of experiments*. New York: Wiley.

Montheard, J.P., Chatzopoulos, M., Chappard, D., 1992. 2-Hydroxyethyl methacrylate (HEMA): Chemical properties and applications in biomedical fields. *J M S – Rev Macromol. Chem. Phys.* C32, 1–34.

Morita, S., 2014. Hydrogen-bonds structure in poly(2-hydroxyethyl methacrylate) studied by temperature-dependant infrared spectroscopy. *Front Chem*. 2.

Pereira, C.S., Cunha, A.M., Reis, R.L., Vázquez, B., San Román, J., 1998. New starch-based thermoplastic hydrogels for use as bone cements or drug-delivery carriers. *J Mater Sci: Mater M*. 9, 825–833.

Thormann, U., Ray, S., Sommer, U., et al., 2013. Bone formation induced by strontium modified calcium phosphate cement in critical-size metaphyseal fracture defects in ovariectomized rats. *Biomaterials*. 34, 8589–8598.

Van Wazer, J.R., Lyons, J.W., Kim, K.Y., Colwell, R.E., 1963. Viscosity and flow measurement: a laboratory handbook of rheology. New York-London: Interscience Publishers.

Wold, S., Ruhe, A., Wold, H., Dunn, W.J III, 1984. The collinearity problem in linear regression. The partial least squares approach to generalized inverses. *SIAM J Sci Stat Comput.* 5, 735–743.

Yu, L., Li, Y., Zhao, K., et al., 2013. A novel injectable calcium phosphate cement-bioactive glass composite for bone regeneration. *PLoS ONE.*8, e62570.