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Modeling of drug release from nanophase hydroxyapatite carriers – statistics in action

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Abstract

Mathematical modeling of drug release from a biomaterial, with respect to time and other factors assumes paramount importance in the field of drug delivery. In this study, doxycycline containing hydroxyapatite ceramics, placed in physiological saline for 10 days, was taken as a model. Numerical equations were derived and curve fitting was done with the observed values. The relations between various parameters and release curve were studied. The observations were verified numerically. The results of the study showed that the drug release can be quantified predictably and controlling the release is possible. Future scope of the study is also discussed.

Keywords: Modeling, Curve-fitting, Doxycycline, Drug delivery, Hydroxyapatite, Spectrophotometry

Introduction

The biomedical sciences owe their major developments to mathematics for quantitative explanation. Mathematical model becomes the proof of an observation. Local drug delivery is a fascinating frontier of biomaterials science, involving a lot of mathematics.

Delivering the drug to the necessary site of action, called local drug delivery, is the most advantageous method in drug delivery, because it is accompanied by lower side effects. Recently, numerous materials have been used for drug delivery, for various purposes. Most of them carry either an antibiotic or an anticancer drug (1).

Local delivery frequently requires surgery to carry the biomaterial into the proper position in the body. Hence, prediction of drug release assumes paramount importance, which is important to design a predictable system.

The basic concept is that, when the drug containing porous vehicle/biomaterial is placed in the body, it will allow diffusion of drug into the target site, following fick's law, which states that the flow occurs from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (spatial derivative). (2) Even though the diffusion mechanism plays a major role, many other factors do have their own influences on the system. For instance, binding of drug to biomaterial can substantially reduce drug release. Solubility of drug in the body fluids also has pronounced influence. The list of these factors is virtually endless.

But, considering a particular drug with one particular biomaterial, most of the factors, like binding, pore size, solubility, etc, are constants. This provides us ample scope to model the drug release. In order to study the drug release, phosphated buffered saline (PBS) is commonly used in place of living body. Such an assessment of drug release in PBS provides a definitive idea of release in the body (3).

Doxycycline is one of the most commonly used antimicrobial agents in bone infections. It is a light-yellow crystalline granule and has a low affinity/attraction for calcium. It is highly stable in normal human tissue fluids. Treatment with doxycycline has been shown to significantly reduce the disease causing microbial population in the oral cavity (4,5)

Calcium phosphate-based bioceramics has been used in medicine and dentistry for nearly 30 years. In recent years Hydroxyapatite (HA) and tricalcium phosphate (TCP) have been used as drug carriers for a wide range of applications. The biocompatibility of these ceramics being excellent (6), they are ideal material to function as carriers for drugs (7). Of these materials, hydroxyapatite has shown higher acceptability. (8)

As observed in many cases, change in size of particles from microsize to nanosize improves biocompatibility (9). Similarly, surface chemistry also influences drug release. The effect of modification of surface chemistry has been studied by María Vallet-Regí et al. (10).

Present study aims at comparing the drug release profile of micro and nano-sized ceramics containing different known quantities of doxycycline, and to provide a mathematical equation which can be used to predict drug release. This can be applied for controlled drug release applications in clinical condition. Also, such an equation should take into consideration virtually all the factors affecting the release.

Previously, Xueyu Chen et al (11) experimented on oral drug administration and the release by the pellets used. They had formulated a master equation for estimating the drug release in the intestines. William J. Addicks et al (12) have numerically evaluated using Laplace transforms for drug release from thin membranes.

To state our aims precisely, we try to answer the following critical questions on mathematical grounds:

1. What is the influence of initial drug present in the drug delivery biomaterial on the duration of release?
2. How do we represent the drug release as a function of time and other factors? Or in other words, how to predict drug release?
3. Does this provide basic criteria for selection of any biomaterial for drug delivery applications?

In the present study, the terms 'micro group' and 'M' refer to the micro-sized ceramic group, while the terms 'nano group' and 'N' refer to the nano-sized ceramic group. IDL represents initial drug loading/composition.

Materials and Methods

Doxycycline used in the study was commercially obtained as Doxycycline Hydrochloride 99.9% (HIMedia, Mumbai, India), in a yellow powder form. The hydroxyapatite of micro size and nano size (<200nm) were purchased from Sigma Aldrich (Bangalore, India). Hydraulic press (Lawrence and Mayo, New Delhi) was used to form the pellets. Phosphated buffered saline was prepared from capsules of PBS (Merck, India). UV visual spectroscopy studies were done using Varian UV–VIS–NIR at 270nm.

Incorporation of Doxycycline and study design

Carefully measured quantities of Doxycycline powder were added to measured quantities (300mg) of ceramic powder, mixed and pelletized under 25 MPa pressures, to obtain the pellet. The samples were broadly divided into 2 groups – Micro ceramic (M) group and Nano ceramic (N) group. Each group consisted of 3 subgroups – loaded with 10mg, 20mg and 30mg, respectively. Each subgroup had a sample size of 10 pellets. In total, 60 pellets were used. Each pellet was 1cm in diameter, 0.2 cm thick.

The experiment

Each pellet was placed in 10ml of PBS (pH 7.2) and stored at 37°C. Absorbance at 270nm in UV visual spectrophotometer was recorded. First absorbance was taken after 1 hour of placement into PBS. Subsequent readings were made at intervals of 24 hours for 10 days. The values were converted to amount of drug in 10ml, (i.e. total drug released in this case), using Beer – Lambert's law (13).

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Absorbance, Ab = E x y x t
Where E is molar absorption constant, y is the concentration and t is the thickness of solution. We observed that 10mg Doxycycline in 10ml solution gave an absorbance of 0.82. Since E and t are constants for all the groups, Ab is directly related to y. Therefore,
Concentration, y = (10 x Ab)/0.82
With this relation, concentrations in terms of mg per 10ml were calculated for all absorbance values observed, which is directly the amount of drug present in the solution.

Data Analysis
Using the obtained concentration and time, graphs were plotted, for which following statistical and mathematical analyses were done. The values from micro and corresponding nano groups were analysed using student t- tests.

The curve fitting was done using FindGraph Ver 2.111 (© UniPhiz Labs) software. The most fitting curve was selected by extrapolating the points to 100 days at steps of 0.25 days using WZGrapher (Ver 0.95, © Walter Zorn, www.walterzorn.com) software. The most fitting curve was selected based both on lowest standard error and the maximum fit of the curve. Derivatives were calculated using WZGrapher software. Curve derivatives (dy/dx or y') till 50 days at step of 0.25 days were calculated for concentration values for every sample.

Results

The drug release profile is shown in table 1 for all the subgroups. The corresponding concentration of drug in 10ml of PBS is shown in table 2. The paired t–test for micro and corresponding nano did not show statistical difference (table 3).

Table 1. The mean absorbance (n = 10) (standard deviation in paranthesis)

Day	N10	N20	N30	M10	M20	M30
1	0.13 (0.0016)	0.14 (0.054)	0.15 (0.0016)	0.13 (0.0007)	0.14 (0.0012)	0.16 (0.0075)
2	0.50 (0.0012)	0.52 (0.0017)	0.54 (0.0106)	0.50 (0.0010)	0.53 (0.0388)	0.53 (0.0362)
3	0.51 (0.0102)	0.54 (0.0040)	0.57 (0.0050)	0.52 (0.0005)	0.55 (0.0092)	0.56 (0.0249)
4	0.67 (0.0324)	0.63 (0.0391)	0.65 (0.0337)	0.64 (0.0159)	0.63 (0.0069)	0.65 (0.0035)
5	0.72 (0.0331)	0.69 (0.0473)	0.70 (0.0495)	0.67 (0.0276)	0.67 (0.0036)	0.72 (0.0210)
6	0.72 (0.0331)	0.69 (0.0471)	0.71 (0.0512)	0.68 (0.0232)	0.68 (0.0009)	0.73 (0.0221)
7	0.74 (0.0357)	0.70 (0.0428)	0.73 (0.0272)	0.69 (0.0159)	0.70 (0.0067)	0.73 (0.0211)
8	0.75 (0.0423)	0.70 (0.0366)	0.76 (0.0030)	0.70 (0.0008)	0.72 (0.0121)	0.74 (0.0218)
9	0.77 (0.0252)	0.73 (0.0331)	0.82 (0.0080)	0.72 (0.0200)	0.78 (0.0200)	0.78 (0.0228)
10	0.77 (0.0247)	0.79 (0.0486)	0.83 (0.0102)	0.74 (0.0236)	0.81 (0.0086)	0.81 (0.0094)

Table 2. Mean concentration of Doxycycline (in mg/10ml, n = 10) (standard deviation in paranthesis)

Day	N10	N20	N30	M10	M20	M30
1	1.60 (0.02)	1.75 (0.07)	1.86 (0.02)	1.59 (0.01)	1.65 (0.01)	1.91 (0.09)
2	6.11 (0.01)	6.39 (0.02)	6.61 (0.13)	6.05 (0.01)	6.42 (0.47)	6.46 (0.44)
3	6.24 (0.12)	6.58 (0.05)	6.93 (0.06)	6.32 (0.01)	6.65 (0.11)	6.85 (0.30)
4	8.13 (0.39)	7.65 (0.48)	7.88 (0.41)	7.82 (0.19)	7.64 (0.08)	7.98 (0.04)
5	8.77 (0.40)	8.36 (0.58)	8.58 (0.60)	8.17 (0.34)	8.17 (0.04)	8.75 (0.26)
6	8.79 (0.40)	8.41 (0.57)	8.62 (0.62)	8.26 (0.28)	8.28 (0.01)	8.85 (0.27)
7	8.97 (0.44)	8.48 (0.52)	8.89 (0.33)	8.37 (0.19)	8.55 (0.08)	8.93 (0.26)
8	9.18 (0.52)	8.57 (0.45)	9.23 (0.04)	8.59 (0.01)	8.79 (0.15)	8.98 (0.27)
9	9.42 (0.31)	8.88 (0.40)	10.03 (0.10)	8.76 (0.24)	9.55 (0.24)	9.46 (0.28)
10	9.43 (0.30)	9.63 (0.59)	10.15 (0.12)	9.01 (0.29)	9.86 (0.11)	9.89 (0.11)

Table 3. The paired t – test (2 tails) for corresponding micro and nano groups (t(Critical) (at 5% error) value:2.262157)

Day	N10 Vs M10	N20 Vs M20	N30 Vs M30
1	0.387164	0.463744	0.308319
2	0.007419	0.377801	0.518757
3	0.395124	0.398563	0.624564
4	0.293468	0.411803	0.744776
5	0.292209	0.434355	0.535579
6	0.304215	0.422419	0.395681
7	0.234792	0.393541	0.472775
8	0.192382	0.388107	0.195560
9	0.155592	0.329042	0.058341
10	0.203402	0.391907	0.001294

Curve fitting was done for every sample to obtain the respective values of a, b and c. In order to find the relation of a, b, c and the IDL, correlation and regression were analysed between all these terms.

The general equation proposed from the analysis is:

$$y = a + b\left(\frac{e^{cx-1}}{c}\right) \quad (1)$$

where x is time in days, y is concentration (amount of drug in 10ml) and a, b and c are constants. The median of values of constants for every subgroup is shown in table 4. These are used to assess the calculated and observed values later. Also, their correlation was analysed, which showed high correlation between a, b and c. But the correlation was very weak for these values to IDL (Table 5).

Table 4. The table of constants for every group (Medians)

Subgroup	a	b	c
N10	-4.67903	8.652015	-0.6145
N20	-4.56199	8.936809	-0.65216
N30	-4.10696	8.480774	-0.62108
M10	-5.63766	10.57454	-0.74091
M20	-3.73765	7.689003	-0.5975
M30	-3.91443	8.128761	-0.61112

Table 5. The correlation and regression of a, b, c and IDL

Parameter	Regression equation	Correlation Coefficient (r)	Coefficient of determination (r^2)	Inference
a on IDL	$a = -3.7806 - 0.1540 (IDL) + 0.004979 (IDL)^2$	0.2999	0.1264	Weakly positive
c on a	$c = -0.4304 + 0.01796 a - 0.006459 a^2$	0.9681	0.9601	Strongly Positive
b on a	$b = 4.0462 - 0.5650 b + 0.1092 b^2$	-0.9855	0.9877	Strongly Negative
c on b	$c = -0.2343 - 0.04368 b - 0.0003084 b^2$	-0.9891	0.9787	Strongly negative

The derivatives were calculated as mentioned. Drug release followed till 36 – 39 days as shown elaborately in table 6, which also shows the final concentration of drug. The statistical analyses of observed and calculated values of released drug are presented in table 7, which shows that there is no significant difference at 5% error.

Table 6. Derivatives table (theoretical and observed)

Group	Time when y' = 0	Final concentration at 10 days	
		Calculated (mg/10ml)	Observed (Mean) (mg/10ml)
N10	~36 days	9.37	9.43
N20	~37 days	9.15	9.63
N30	~39days	9.52	10.15
M10	~36 days	8.40	9.01
M20	~38 days	9.09	9.86
M30	~38 days	9.35	9.89

Table 7. Paired t-test (2 tails) at 5% error for observed and calculated values ($t_{critical}$ = 2.262)

Parameter	N10*	N20*	N30*
P(T<=t)	0.294681	0.010299787	2.84E-08
Parameter	M10*	M20*	M30*
P(T<=t)	1.84E-05	4.15E-10	2.78E-08

*H_0 = True (Observed and expected values are statistically same)

Figure 1: Drug release profile

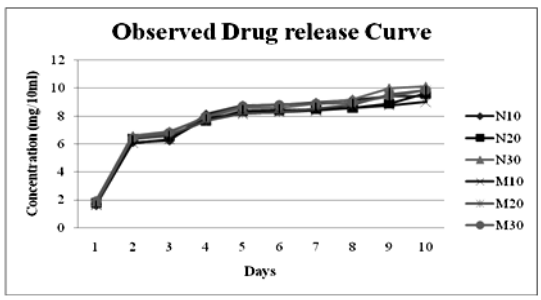
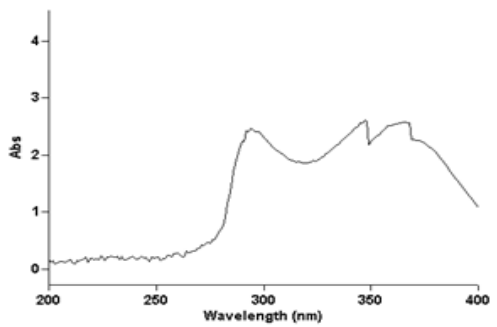


Figure 2. Curve of doxycycline hydrochloride solution in PBS



Discussion

The general observed release profile is shown in figure 1. The doxycycline hydrochloride studied under UV-Visible spectroscopy

revealed characteristic spectrum of the drug. It had 2 peaks in the UV region (Fig 2) (270nm and 348nm) and prominent peak at 270 nm was used to analyse drug release profiles.

As observed, prior to the mathematical analysis, the results are as follows. Micro ceramic pellets with 10mg of doxycycline stopped release in 6 days, whereas nanoceramic with 10mg stopped release only on the 7th day. This shows that nanoceramic exhibits longer duration of release than the micro ceramics. More doxycycline retention by microceramic than nanoceramic is clearly indicated by the absorbance for microceramic (0.72 on 10th day) and the absorbance for nanoceramic (0.77 on 10th day).

The absorbances presented in table 1 are the mean values of that respective subgroup. Table 2 presents the amount of drug present in 10ml of PBS. Statistical testing of respective M and N groups showed conclusively that they were similar (Table 3). Hence it may be tempting to conclude that the release of Doxycycline occurs irrespective of particle size, implicitly pore size. But, compression by hydraulic press (25MPa) during pelletization can reduce the inter-particle space, that is, the pore size. Thus this similarity can be attributed to compression.

Under curve fitting procedure, as mentioned above Findgragh software was used. It had the capability to accept coordinates/points and plot curves using simplex, gradient, and Levenberg - Marquardt algorithms. It provided the equation, constants and standard error for every curve it plot. Complete visualization of the curve was done with WZGrapher, using the equation provided by the Findgraph. The curve with the best fit was selected based on lowest residuals. A few other curves showed lesser standard error, but they did not fit the data in the extrapolation, that was done using WZGrapher. That is, the curves were either parabolic, resulting in reduction of concentration with time, say, after 30 days, etc, or were exponential curves, in which the concentrations kept increasing and never came to a constant, contrary to the results of the study. Hence present equation is selected as the apt curve, using visualization by WZGrapher software. The 3 constants are independent of time and concentration of doxycyline in the solution, but may be related to other factors like IDL, pH, temperature, etc. Table 4 lists the median of these constants for every subgroup. Since it is intended to give an accurate equation, we wanted to eliminate the influence of extremes of values, which might cause higher error in the prediction. So medians are used and not means.

To analyse this relationship, the constants were checked for correlation and regression, which showed that a, b and c are strongly correlated as shown in the table 5. Both first order and second order regression were calculated. The second order regression equation showed lesser residuals than the first order. This relation shows any variable influencing 'a' will have definite negative influence on 'b' and positive influence on 'c'. But correlation of 'a' to IDL was weakly positive (0.2999). Coefficient of determination shows that only 0.1264% of variability in 'a' is accounted by IDL. This means that there are other strong influencing factors for drug release. Similarly coefficients of determination between a, b and c are also very small, implying almost insignificant relation between them (~1%), or in other words they are independent of each other. Other factors influencing drug release would be pH and temperature as stated. In a physiological condition, these two are irrelevant since they are constants. Probably, other factors like dissolution of hydroxyapatite can play a major role. It was seen that the pellets were recovered as a whole at the end of the study. Hence the analysis did not include degradation into the criteria.

Differentials analysed for 50 days showed that all the pellets, irrespective of initial loading, stopped release from 36 – 39 days (Table 6). This means, initial drug composition does not have a major role. As analytically observed, increase in initial loading prolonged the release by one day.

The reason for 20mg and 30mg groups to stop the release of the drug at ~10mg level might be saturation of the solution. But, the notable fact is, till the saturation, it followed the same equation. In the living body, such saturation cannot occur and pellets will continue to release the drug. But, the amount retained by the 10mg groups is significant and that cannot be expected to be released, unless the pellet is degraded. This retention value is almost constant. The study had a time limitation of 10 days. Perhaps, the extension of study period can shed more light. But primarily, the first 5-10 days are of prime importance to the surgeon, to eliminate the infection. When such a pellet is implanted within the bone, bioavailability will be 100% and hence, even if the entire drug does not get released, the pellet will not harbour infection. Since the pellet in bone is not expected to release the drug as it would in a hydrated soft tissue, longer antimicrobial activity can be expected.

Also, the observed and calculated mean values at 10 days were computed to verify the fit of the proposed equation. Paired student's t-test (2 tails) for calculated and observed values (H_0 = there is no statistical difference between observed and calculated values), showed that they were not statistically different at 5% error (α=0.05) (Table 7). This means that the equation successfully predicts the release profile of doxycycline loaded hydroxyapatite at a maximum of 5% error.

This analysis has also illuminated the concepts of evaluation of a biomaterial as a vehicle. If the curve is shorter in height than the expected values, the material does not warrant selection as a vehicle.

In future, the parameters affecting a, b and c are to be evaluated. The relation of pelletization pressure to drug release is also being evaluated. Also, in vivo trials are underway and will be reported when results are available.

Conclusion

This study has shed valuable light on the application of mathematics to predict the drug release. A mathematical equation was successfully proposed and tested based on statistical methods.

The answers to the three questions are:

1. Initial drug loading has apparently no influence on drug delivery.
2. The drug release has been successfully represented as a function of time.
3. Basic criteria for selection of any biomaterial for drug delivery applications are discussed.

Also, nanoceramic is found to be marginally better than microceramics in duration, rate of release and effective drug availability in the current scenario. We could not find the influence of particle size on drug release due to the pelletization technique used. It was found that micro ceramic retained more drug than nanoceramic, both by the mathematical equation and the experiment. The derivative of the curve reduced progressively which indicated that the release progressively reduced until zero. The three constants given in the equation are strongly correlated. The constants and the IDL in pellet were only weakly positively correlated, strongly suggesting the influence of other factors like pH, temperature, degradation of materials, etc. It is clearly seen that increase in IDL will lead to longer periods of release and not higher levels at short time.

Thus this study has showed that, statistical methods can be successfully applied to model biological phenomena.

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