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ORIGINAL ARTCLE / ÖZGÜN MAKALE

DEVELOPMENT OF *IN SITU* GEL FORMULATION CONTAINING BISPHOSPHONATE-LOADED PLGA MICROSPHERES FOR BONE REGENERATION IN MAXILLOFACIAL SURGERY APPLICATIONS; FORMULATIONS, *IN VITRO* CHARACTERIZATION AND RELEASE KINETIC STUDIES

MAKSİLLOFASİYAL CERRAHİ UYGULAMALARINDA KEMİK REJENERASYONU İÇİN BİFOSFONAT YÜKLÜ PLGA MİKROKÜRELERİ İÇEREN İN SİTU JEL FORMÜLASYONLARININ GELİŞTİRİLMESİ; FORMÜLASYONLAR, İN VİTRO KARAKTERİZASYON VE SALIM KİNETİK ÇALIŞMALARI

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ABSTRACT

Objective: *In this study, it was aimed to locally apply the bisphosphonate-loaded microsphere drug delivery system with in situ gel formulation, which was prepared to increase bone regeneration in the implant area in maxillofacial surgery.*

Material and Method: *In order to design the combination delivery system, bisphosphonate-loaded PLGA microspheres were embedded in the prepared in situ gel formulations. In vitro drug release, pH, clarity, sol-gel transition temperature and release kinetic studies were all assessed for the developed formulations.*

Result and Discussion: *The produced formulations' in situ gelation temperatures ranged from 33 to 37°C; their pH values were in the range of 6; and they were all syringeable, which is defined as the force required to expel each formulation from a syringe equipped with a 20-gauge needle. With the preparations, the amounts of*

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P407 and chitosan increased, lowering in vitro burst release while simultaneously raising viscosity. However, each in situ gel formulation releases over a period of 14 days. Consequently, Bisphosphonate loaded PLGA microspheres embedded in situ gel formulations were elucidated in detail and presented as a locally applicable drug delivery system in maxillofacial surgery, especially in dental implant applications

Keywords: *Bisphosphonate, combination delivery systems, plga microspheres, poloxamer, chitosan*

ÖZ

Amaç: *Bu çalışmada maksillofasiyal cerrahide implant bölgesinde kemik rejenerasyonunu artırmak için hazırlanan bifosfonat yüklü mikrosfer ilaç taşıyıcı sistemin in situ jel formülasyonu ile lokal olarak uygulanması amaçlanmıştır.*

Gereç ve Yöntem: *Kombinasyon taşıyıcı sistemini tasarlamak için bifosfonat yüklü PLGA mikroküreleri, hazırlanan in situ jell formülasyonlarına yüklenmiştir. Geliştirilen formülasyonlar için in vitro ilaç salım, pH, berraklık, sol-jel geçiş sıcaklığı ve salım kinetik çalışmaları değerlendirilmiştir.*

Sonuç ve Tartışma: *Üretilen formülasyonların yerinde jelleşme sıcaklıkları 33 ila 37°C arasında; pH değerleri 6 civarında ve bütün formülasyonlar 20 gauge'lik şırıngalardan uygulanabilir düzeydeydi. Preparatlar içerisinde yer alan, P407 ve kitosan miktarları arttıkça, in vitro patlama salınımını düşürürken aynı zamanda viskoziteyi yükselmiştir. Bununla birlikte, her bir in situ jel formülasyonu, 14 günlük bir süre içinde salım yapmıştır. Sonuç olarak, Bifosfonat yüklü PLGA mikroküreleri yüklü in situ jel formülasyonlarına ayrıntılı olarak değerlendirilmiş ve özellikle dental implant uygulamalarında maksillofasiyal cerrahide lokal olarak uygulanabilir bir ilaç taşıma sistemi olarak sunulmuştur*.

Anahtar Kelimeler: *Bifosfonat, kombinasyon taşıma sistemleri, plga mikroküreler, poloksamer, kitosan*

INTRODUCTION

Due to the restricted amount of bone available for implant placement and the postoperative bone's mechanical characteristics, bone regeneration is a critical component of maxillofacial surgery, particularly in implantology procedures increased osteoclastic activity, which accelerates bone resorption, may be the root cause of low bone density at the surgical site and the associated clinical implant failure [1]. In order to address peri-implant bone abnormalities, particularly for maxillofacial processes, preclinical and clinical investigations have concentrated on novel therapeutic approaches using multidisciplinary concepts [2]. Working together in disciplines like medicine, pharmaceutical sciences, biology, and materials science can help create effective and novel approaches to bone regeneration because it is an interdisciplinary and complicated process.

According to their affinity for hydroxyapatite, bisphosphonates are strong inhibitors of osteoclastic bone resorption and can improve bone density by shifting the ratio of bone production to bone resorption [3, 4]. The U.S. Food and Drug Administration (FDA) has approved a class of third-generation bisphosphonates called alendronate sodium (AS), which is well known for preventing bone loss and boosting bone mass [5, 6]. Because of bone resorption, it is sometimes difficult to employ bisphosphonates, which include AS, to repair bone abnormalities, especially in maxillofacial surgery. These issues are still being researched as current pharmaceutical issues [7].

Innovative polymeric micro drug delivery systems that can be applied locally to the site of the bone defect administer medications with a controlled release while acting as mechanical supports for

the region where bone resorption takes place. Research is still being done on methods to improve AS's stability and duration spent in the resorption zone, prolong the drug's release, and lessen side effects. Due to the aforementioned problems, AS is rarely used, particularly in procedures involving dental implants and maxillofacial surgery [8, 9].

One of the most popular synthetic biodegradable polymers in the pharmaceutical industry, Poly(lactic-co-glycolic acid) (PLGA), has received FDA and European Medicines Agency approval (EMA [10]. It is frequently employed in the creation of novel drug delivery systems (NDDS), biomedical and bioengineering applications due to its physiological inertness, biocompatibility, and biodegradability [11]. Problems with solubility and associated bioavailability of active ingredients can be overcome with the use of micro/nanoparticulate drug carrier systems comprised of PLGA copolymers. As a result, therapeutic effectiveness and side effects related to dose can be improved. Additionally, release patterns may be changed, dosage frequency may be decreased by controlled or delayed release, and the required dose may be reduced by increasing the molecule's cellular interaction [12].

The periodontal pocket, the intended location for local drug delivery, is a complicated area with a continuous flow of saliva fluid. To deliver the drug's intended effects in the challenging environment, an injectable *in situ* sustained release gel would be the rational and effective choice. An innovative technique being used at the moment to do this is the use of in-situ gel-forming formulations, which first supply drugs in a liquid dosage form before producing powerful gels at the delivery site. Among in-situ gelling polymers, thermosensitive systems like poloxamer have been researched as an acceptable dosage form for injection into periodontal pockets. In order to improve contact intimacy and lengthen the residence time of the dose form in the periodontal pocket, semisolid formulations containing mucoadhesive polymers such, hydroxyethyl cellulose (HEC), chitosan, and carbopol have also been proposed [13].

The objective of the current study is to prepare and evaluate the *in situ* gel formulation containing Bisphosphonate-loaded PLGA microspheres (BMA) (22) as a local medication with combination delivery system approach for bone regeneration in maxillofacial implant operations. These formulations contained chitosan, a mucoadhesive polymer, as well as the thermosensitive polymer poloxamer 407 (P407). For this purpose, AS loaded PLGA microspheres prepared and published within the scope of our previous study were used [14]. The present study is designed as a further study inspired by the results of the previous study. Promising results of PLGA microspheres prepared with AS, which is widely used in bone regeneration in order to prevent post-operative bone defect and bone loss in the implant area in maxillofacial surgery applications, have been extensively reported in our previous study. In this study, an *in situ* gel formulation, which allows easy application of AS-loaded PLGA microspheres

to the implant site where bone regeneration is desired, prolongs the residence time of the formulation and drug release, was developed and *in vitro* characterization studies were completed.

MATERIAL AND METHOD

Materials

Poly(lactide-co-glycolide) (PLGA) 50:50 (RG 503H), alendronate sodium trihydrate (solubility in water 10 mg/mL), poloxamer, chitosan (low molecular weight), phosphate buffered saline (PBS) tablet were purchased from SigmaAldrich.

Method

Preparation and *in vitro* characterization of BMA formulation

Within the scope of this study, AS loaded PLGA microsphere formulations embedded in gel formulations were prepared in our previous study and comprehensive *in vitro* characterization studies were completed and reported [14]. In summary, PLGA microspheres were prepared by the multiple emulsion method (W/O1/O2) and characterization parameters, morphology, release profiles, degradation studies, stability and *in vitro* release kinetics were analyzed. It was observed that the AS release could be maintained for 14 days with the prepared drug delivery system, and PLGA microspheres deteriorated over time during this period. The release kinetics of the microspheres were mathematically modeled and found to be compatible with the Weibull and Peppas-Sahlin models [14].

In conclusion, the *in vitro* properties of AS loaded PLGA microspheres, which can be used in maxillofacial surgery applications, were elucidated in detail and presented as a locally applicable microparticulate drug delivery system for dental bone regeneration. In the light of the results obtained, it is aimed to design an *in situ* gel formulation that can be applied locally to the effect area with an advanced formulation approach for the microsphere formulation, which was designed and characterized. Comprehensive *in vitro* characterization data of AS-loaded PLGA microspheres prepared within the scope of the previous study and forming the basis of this study's details are available in our previous study [14].

Preparation of the In Situ Forming Gels

The *in situ* gel formulations were prepared using a modified cold technique [15]. The polymer was combined with cold (4 °C) water to create all of the P 407 solutions (17, 19 and 21 % w/v) utilized in this work. For 24 hours, the polymer solutions were refrigerated. The chitosan (0.5, 1% w/v) solution was then made ready for formulations. Chitosan was first dissolved in a 2 % v/v acetic acid solution, after which the chitosan solution was refrigerated for 24 hours. The chitosan solution and PF127 solution

were then mixed together at 4 °C then 0.2% BMA was added to the solution. The samples are still kept at 4 ° C in preparation for auxiliary exercise.

Table 1. Ingredients of *in situ* gels

	Content of ingredients in each formulation (%, w/w)			
Code	**Poloxamer 407**	**BMA**	**Chitosan (low molecular weight)**	**Water (qs)**
A1	17	0.2	0.5	100
A2	17	0.2	1	100
A3	19	0.2	0.5	100
A4	19	0.2	1	100
A5	21	0.2	0.5	100
A6	21	0.2	1	100

pH

Using a pH meter, the pH was determined. (HANNA of Germany) A total of three measurements were made (n=3).

Clarity

After gelation, the formulation of the *in situ* gel was examined in bright light against a dark background to determine its clarity [16].

Syringeability study

The ability of the created formulations to flow easily through a syringe with a 20 gauge needle was assessed using the same method as Maheshwari et al. [17]. Before assessing the flowability of the cold gel at standard handling pressure, one cc of the gel was placed into a 20 gauge needle syringe.

Gelation Temperature

In a water bath, a magnetic stirrer was used to stir each polymer solution (10 ml). At a speed of 100 rpm and 1 °C/min, heated polymer solutions were swirled (Thermomac-TM19). Three measurements were made on each [18].

Viscosity

The viscosity of *in situ* gels was measured using a CP 52 spindle and the Brookfield, DV2T-RV viscometer (Essex, UK). Additionally offered for comparison was viscosity at 10 rpm. Viscosities of all *in situ* gels were measured at both 25°C and 37°C (Table 3). Three measurements were made on each [19].

In Vitro Release Studies

The release of *in situ* gel compositions *in vitro* was examined using the dialysis bag method. [20]. The next procedure was to seal the dialysis bags, add 100 µL of BMA loaded *in situ* gel, and then place them at 37 °C in 25 mL of an isotonic phosphate buffer with a pH of 7.4. Such is the provision of the sink condition. Equivalent amounts of the medium were removed and replaced with equivalent amounts of the new buffer media at various intervals (0, 0.5, 1, 2, 4, 7, 10, 12, and 14 days). BMA concentrations were measured using a UV-vis spectrophotometer. To create a BMA release profile, the total amount of medicine released from each formulation over time was used. Three times were run through the experiment.

Release Kinetic Studies

DDSolver software was used to evaluate the *in vitro* release kinetic of AS from *in situ* gel formulations. In this regard, various mathematical models were used to examine the kinetics of drug is released from *in situ* gel formulations (Zero order, First order, Higuchi, Korsmeyer-Peppas, Peppas-Sahlin and Weibull). In order to select the "best fit" models, data were computed using the DDSolver program after evaluating the *in vitro* AS release profiles from formulations. Four criteria were evaluated; Akaike Information Criterion (AIC), coefficient of determination (R^2), adjusted coefficient of determination (R2adjusted), and model selection criterion (MSC). The greatest R^2, $R^2_{adjusted}$, MSC, and lowest AIC values were used to choose the model that best fitted the *in vitro* release data and had a higher correlation [21].

RESULT AND DISCUSSION

Preparation and *in vitro* characterization of BMA formulation

The characterization findings of AS loaded PLGA microspheres, which were prepared in our previous study and whose comprehensive *in vitro* characterization studies were completed, and embedded in the *in situ* gel formulations prepared in this study, was briefly explained in the method section. Details are available in our previous study [14].

Characterisation of *in situ* gel formulations

The viscosity results of blank *in situ* gels are shown in table 2. Viscosity coefficients were measured at 10 rpm at both 25 °C and 37 °C for A1-A6. When compared with microsphere loaded *in situ* gels, it is seen that there is no significant difference ($p<0.05$).

All of the *in situ* forming gel formulations for BMA were found to gel between 31 and 37 °C, making them all appropriate for use in dental procedures. Table 3's data serve as evidence. All

formulations have a pH that ranges from 5.92 to 6.11. It can be syringed using a 20 gauge needle, according to research.

Table 2. Results of *in situ* gels' *in vitro* characterisation analysis

Formulation	Viscosity (centipoise) 25 °C	Viscosity (centipoise) 37 °C
A1	240±34	8904±102
A2	282±29	8994±154
A3	300±17	9187±297
A4	308±25	9372±203
A5	315±24	9888±302
A6	331±28	10057±197

As a result, viscosity coefficients were measured at 10 rpm at both 25 °C and 37 °C for all formulations. The findings demonstrated that the values of viscosity varied according to the amounts of polymer (Table 3).

Table 3. Results of *in situ* gels' *in vitro* characterisation analysis

Formulation	pH (±SD)	Gelation temperature (°C±SD)	Viscosity (centipoise) 25 °C	Viscosity (centipoise) 37 °C	Clarity
A1	6.11±0.01	37±0.7	231±21	8842±121	Clear
A2	6.02±0.02	36±0.3	275±31	8938±169	Clear
A3	5.99±0.03	35±0.1	290±22	9145±312	Clear
A4	5.95±0.02	34±0.2	301±14	9352±218	Clear
A5	5.93±0.08	33±0.4	314±38	9868±214	Clear
A6	5.92±0.02	33±0.6	326±29	10042±231	Clear

Two different temperatures-room temperature of 25°C and the application periodontal pocket site temperature of 37°C-were used in the sol-gel transition studies to determine if the formulations were suitable for in-situ application as well as storage conditions [22,23]. According on the grade, concentration, and other formulation elements used, poloxamer solutions have been observed to undergo thermoreversible gelation.

When temperatures are lower than the critical micelle temperature (CMT), at which the critical micelle concentration (CMC) occurs, poloxamer maintains each molecule's separation in an aqueous

environment. When the temperature exceeds CMT, the molecules are stimulated to organize into micelles that surround the hydrophobic core while maintaining contact with the aqueous media through hydrophilic pluronic chains. Consequently, a lower CMT value is produced by a higher poloxamer concentration [23]. The gelation temperature is also negatively impacted by this circumstance. The concentration of poloxamer's polymer is mostly associated with the gelation temperature. At lower concentrations, they produce monomolecular micelles, but at greater concentrations, multimolecular lattice structures are produced [24-26].

Our formulations use lower poloxamer concentrations so that gelation happens at corneal temperature. As a result, as the concentration of poloxamer decreased, gelation temperatures increased. The literature turned up similar results [27]. Increasing the chitosan concentration has a detrimental impact on the gelation temperature. P407's hydrophobic domains were aggregated during gelation to lessen the hydrophilic surfaces and the amount of water surrounding the hydrophobic domains [28].

Table 3 displays the *in vitro* characterization findings of *in situ* gels. The look of all formulations was clear. The pH of all formulations ranges between 5.92 to 6.11. Hypodermic syringes with gauges 19–27 are used for oral injection. An extremely viscous solution requires the use of a needle with a smaller gauge [29]. Syringeability is defined as the amount of force necessary to discharge each formulation from a syringe fitted with a 20-gauge needle. The syringeability requirements are met by all formulations.

The formulations with a larger amount of chitosan were found to be more viscous than those with a lower amount, according to the results of the viscosity test. The amount of gel that may be injected into periodontal pockets is extremely constrained, so it must be thickened for improved retention and medication release control. On the other hand, the formulation ought to have the ideal viscosity to easily infuse in the periodontal pocket.

As a result, viscosity coefficients were measured at 10 rpm at both 25 °C and 37 °C for all formulations. The findings demonstrated that the values of viscosity varied according on the amounts of polymer (Table 3). This illustration shows how viscosity is significantly influenced by the concentration of the polymer. When the findings were analyzed in literary context, the conclusions are consistent [30].

Drug Release

Experiments on the *in vitro* release of drugs were conducted on *in situ* gels at 35 °C in an isotonic phosphate buffer with a pH of 7.4 and BMA (% 0.2). *In vitro* release profiles of AS loaded MS and the BMA loaded *in situ* gels were monitored for 14 days (Figure 1).

Comparing release profiles revealed a noticeable variation between them (Fig. 1). The overall release percentage for AS loaded MS was 96 ±3.1%. When BMA loaded (A1-A6) *in situ* gels are examined, it is seen that drug release is between 88± 1.1% and 91%± 1.3. The burst effects of the formulations are clearly shown in Fig.2. It is also seen that the burst release from the microspheres is less with the gel formulation. It indicates that from 0 h to 24 h, the cumulative release percent of AS loaded MS were 31.2± 2.54% and when BMA loaded (A1-A6) *in situ* gels are examined from 0h-24h, it is seen that drug release is between 16.7 ± 0.7% and 19.6±1.2%. In comparison to *in situ* gels, AS loaded MS had a much greater release percentage.

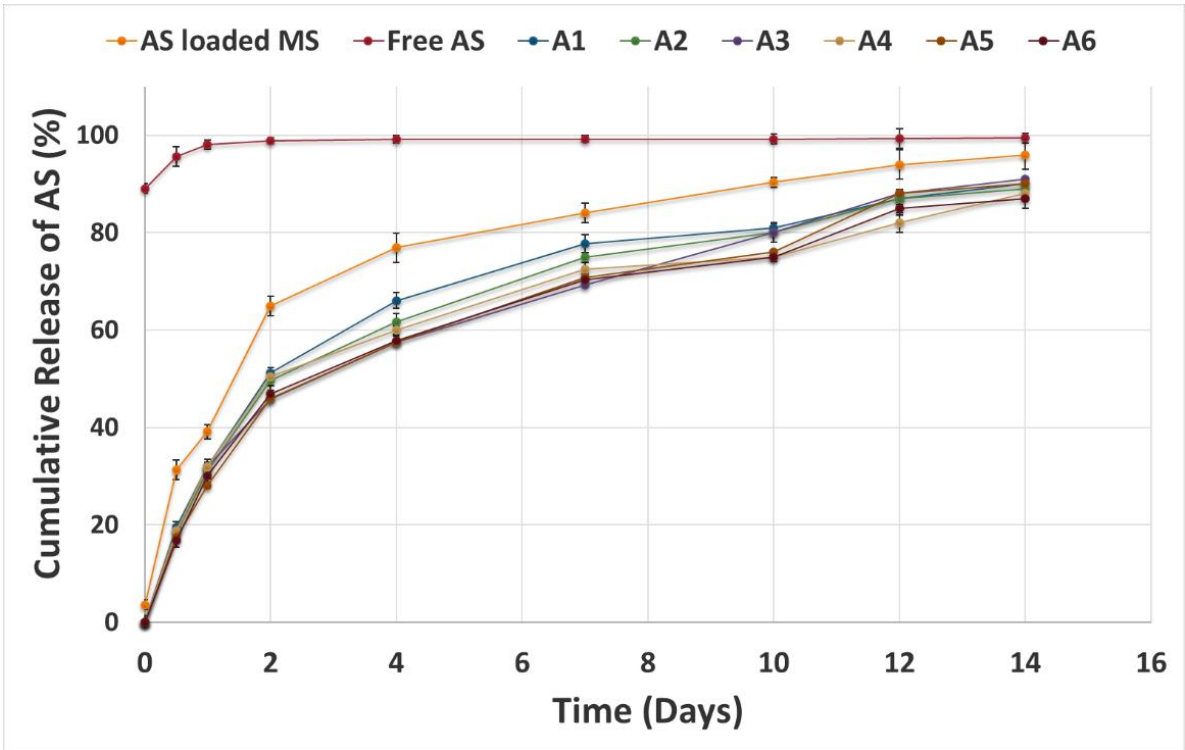

Figure 1. Cumulative release of AS from microspheres and *in situ* gel formulations containing AS-loaded microspheres (A1-A6)

Poloxamer gel deteriorated fully in 24 hours in our earlier erosion investigation, which isn't discussed in this publication. As a result, it is reasonable to assume that *in situ* gel release profiles consisted of two steps: in the first, the *in situ* gel gradually degraded while simultaneously releasing the microspheres from the skeleton carrier. The second phase involved the drug's gradual release from PLGA microspheres. This provided an explanation for the differences between the two release profiles of the two formulations. At first, the gel prevented AS loaded MS from touching the release medium. An increasing number of microspheres discharged as the gel slowly degraded. Because the release behavior of microspheres was dependent on a diffusion mechanism, they all released after the gel had

completely dissolved at 24 hours and did so at a rate that was comparable to that of AS loaded MS. When the literature is evaluated, it can be shown that the findings are consistent [31-35].

When *in situ* gel formulations are examined among themselves, the burst release diminished when poloxamer P407 concentration grew from 17% to 21%. It's possible that fewer and larger water channels and more and larger micelles inside the gel structure are the mechanisms producing this improved resistance. Because of the closer proximity of the micelles, there are more cross-links, which results in a higher viscosity and a slower rate of drug release. This hypothesis might be supported by rheology research, which shows a direct correlation between gel concentration and viscosity [15]

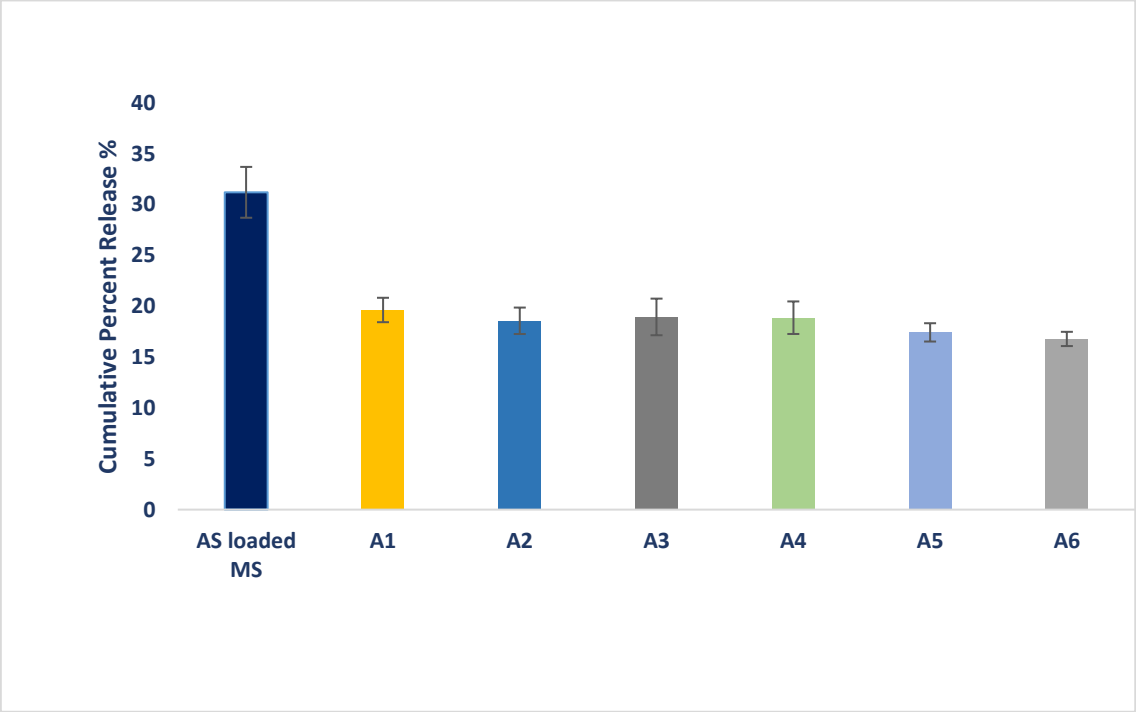

Figure 2. Comparison of *in vitro* AS release from microspheres and *in situ* gels (0h-24h)

Release Kinetic Studies

Various kinetic models were applied mathematically to the formulations in order to examine the AS release mechanism from the prepared combined delivery systems (Zero order, First order, Higuchi, Korsmeyer-Peppas, Peppas-Sahlin, and Weibull). Six different models were applied to six different combination delivery systems and microsphere formulations to examine critical parameters and comprehensively evaluate their release kinetics. Details and results on parameters that are valuable for release kinetic studies are shown in Table 4. As can be seen in the table, higher correlation was found for all formulations in Peppas-Sahlin and Weibull models compared to other models. From this point of view, it was evaluated that the kinetics of AS release from the prepared drug delivery systems fit both

Weibull and Peppas-Sahlin models, and it was seen that the release kinetics fit not only one model but more than one model. These results are consistent with the reports that the release kinetics of new drug delivery systems can fit more than one model and combined release kinetics can be observed, in line with the literature [36]. In the Weibull model, the "β" exponent is a indicator used to evaluate the release from a polymeric structures and "β"≤0.75 indicates Fickian diffusion [37]. Diffusion was considered to be the dominant mechanism in the Weibull model for all formulations. This situation was interpreted as compatible with both the release mechanism from the gel and the release mechanism from the microspheres. Considering the other compatible model, it should be evaluated in terms of the Peppas-Sahlin model. In principle, The Peppas-Sahlin model is based on Fickian diffusion and erosion of the polymeric matrix [38]. Polymeric matrix erosion is important both for microspheres and for release from the gel structure. When evaluated in the light of this information, the Peppas-Sahlin model also supports the diffusion phenomenon associated with erosion of the polymeric structure. In this context, It was observed that there was a mixed process in both the Fickian (pure diffusion phenomena) and non-Fickian models for the release of AS from formulations (due to the relaxation of the polymer chains between the networks). At the end of the release kinetic analysis, it was determined that both diffusion-based Fickian and polymeric structure degradation-based non-Fickian mechanisms were observed with the *in situ* gel combination delivery system.

In conclusions, bone loss in the surgical site caused by bone resorption is still one of the most common clinical consequences, particularly in maxillofacial surgery applications. Pharmaceutical research on the viability of medications that act locally in the surgical site and can stimulate bone growth by preventing bone resorption is ongoing for this purpose. The usage of AS, which is supposed to stop bone loss and increase bone mass, is still ineffective, particularly following dental implant placement. As part of the experiment, various polymer solutions including different concentrations of P407, the mucoadhesive polymer chitosan, and BMA were created. These formulations were all put to the test *in vitro* (pH, clarity, gelation temperatures and syringeability). The gelation temperature dropped when the poloxamer concentrations were compared to the gelation temperatures. However, it was discovered that the pH of all formulations was close to 6. The compositions would not irritate the dentition, according to research. When the drug release of formulations is examined, it is seen that busrt release decreases with increasing concentration. However, when all these *in vitro* characterization studies are evaluated, it is seen that A1 and A2 may be the most appropriate formulation. Since bone resorption is one of the most significant challenges in maxillofacial surgery applications, this formulation can be assessed as a novel medication delivery strategy that may improve bone regeneration in order to overcome the issues associated with them. In light of this, it has been deemed to be a promising drug delivery for upcoming preclinical and clinical studies.

Table 4. Release kinetic modeling and results of AS-loaded PLGA MC and BMA loaded *In Situ* Gels

Model and equation	Formulation		Evaluation criteria							
			Parameter		R^2	$R^2_{adjusted}$	AIC	MSC	n/m*
Zero-order	MS embedded in situ gel formulations	A1	k0	7.970	0.4918	0.4918	77.2040	-0.0160	-
		A2	k0	7.841	0.5470	0.5470	75.9833	0.1168	-
		A3	k0	7.796	0.6313	0.6313	74.0604	0.3398	-
		A4	k0	7.555	0.4957	0.4957	76.0188	-0.0196	-
F=k0*t		A5	k0	7.702	0.6511	0.6511	73.6385	0.4191	-
		A6	k0	7.532	0.5912	0.5912	74.4478	0.2354	-
	AS-loaded PLGA MS		k0	8.732	0.1763	0.1763	81.7092	-0.0282	-
First-order	MS embedded in situ gel formulations	A1	k1	0.265	0.9342	0.9342	58.8002	2.0289	-
		A2	k1	0.255	0.9306	0.9306	59.0998	1.9928	-
		A3	k1	0.261	0.9166	0.9166	60.6840	1.8261	-
		A4	k1	0.231	0.8833	0.8833	62.8467	1.4439	-
F=100*[1-Exp(-k1*t)]		A5	k1	0.252	0.9221	0.9221	60.1488	1.9180	-
		A6	k1	0.232	0.9068	0.9068	61.1379	1.7143	-
	AS-loaded PLGA MS		k1	0.364	0.9261	0.9261	60.0101	2.3828	-
Higuchi	MS embedded in situ gel formulations	A1	kH	26.739	0.9332	0.9332	58.9376	2.0136	-
		A2	kH	26.208	0.9506	0.9506	56.0392	2.3328	-
		A3	kH	25.877	0.9757	0.9757	49.5694	3.0610	-
		A4	kH	25.321	0.9352	0.9352	57.5535	2.0321	-
F=kH*t^0.5		A5	kH	25.534	0.9747	0.9747	50.0353	3.0417	-
		A6	kH	25.087	0.9610	0.9610	53.3071	2.5844	-
	AS-loaded PLGA MS		kH	29.697	0.8366	0.8366	67.1514	1.5893	-
Korsmeyer-Peppas	MS embedded in situ gel formulations	A1	kKP	31.538	0.9620	0.9565	55.8744	2.3540	0.434
		A2	kKP	30.111	0.9704	0.9662	53.4355	2.6221	0.446
		A3	kKP	29.724	0.9904	0.9890	43.2572	3.7624	0.442
		A4	kKP	30.559	0.9689	0.9645	52.9455	2.5441	0.422
F=kKP*t^n		A5	kKP	27.720	0.9812	0.9785	49.3660	3.1161	0.470
		A6	kKP	28.072	0.9738	0.9700	51.7317	2.7594	0.458
	AS-loaded PLGA MS		kKP	42.812	0.9622	0.9568	55.9752	2.8311	0.336
Peppas-Sahlin	MS embedded in situ gel formulations	A1	k1	**40.079**	**0.9851**	**0.9802**	**49.4194**	**3.0712**	**0.450**
		A2	k1	**37.309**	**0.9881**	**0.9841**	**47.2227**	**3.3125**	**0.450**
		A3	k1	**33.461**	**0.9953**	**0.9937**	**38.8371**	**4.2535**	**0.450**
		A4	k1	**37.571**	**0.9858**	**0.9811**	**47.8819**	**3.1067**	**0.450**
F=k1*t^m+k2*t^(2*m)		A5	k1	**32.265**	**0.9896**	**0.9862**	**45.9934**	**3.4908**	**0.450**
		A6	k1	**34.040**	**0.9879**	**0.9839**	**46.7403**	**3.3140**	**0.450**
	AS-loaded PLGA MS		k1	**52.786**	**0.9857**	**0.9809**	**49.2549**	**3.5778**	**0.450**
Weibull	MS embedded in situ gel formulations	A1	β	**0.604**	**0.9973**	**0.9964**	**34.0547**	**4.7784**	-
		A2	β	**0.609**	**0.9975**	**0.9966**	**33.2835**	**4.8612**	-
		A3	β	**0.610**	**0.9957**	**0.9959**	**37.9087**	**4.6900**	-
		A4	β	**0.565**	**0.9929**	**0.9906**	**41.5984**	**3.8049**	-
F=100*{1-Exp[-((t-Ti)^β)/α]}		A5	β	**0.632**	**0.9922**	**0.9896**	**43.4439**	**3.7741**	-
		A6	β	**0.603**	**0.9944**	**0.9925**	**39.8409**	**4.0806**	-
	AS-loaded PLGA MS		β	**0.566**	**0.9910**	**0.9881**	**45.0154**	**4.0489**	-

Best fit release kinetic models shown with gray fill; In all models, F is the fraction (%) of drug released in time t, k0: zero-order release constant, k1: first-order release constant, kH: Higuchi release constant, kKP: release constant incorporating structural and geometric characteristics of the drug-dosage form, n: is the diffusional exponent indicating the drug-release mechanism, m: diffusional exponent and similar exponent like ‘’n’’, m use in Peppas-Sahlin model equation only, β: the shape parameter which characterizes the curve as either exponential (β=1; case 1), sigmoid, S-shaped, with upward curvature followed by a turning point (β > 1; case 2), or parabolic, with a higher initial slope and after that consistent with the exponential (β < 1; case 3). Values shown in bold and with gray fill in the table are selections made according to criteria.

AUTHOR CONTRIBUTIONS

Conception: *H.K.P., S.Ü.*; Design: *H.K.P., S.Ü.*; Supervision: *H.K.P., S.Ü.*; Resources: *H.K.P., S.Ü.*; Materials: *H.K.P., S.Ü.*; Data Collection and/or processing: *H.K.P., S.Ü.*; Analysis and/or interpretation: *H.K.P., S.Ü.*; Literature search: *H.K.P., S.Ü.*; Writing manuscript: *H.K.P., S.Ü.*; Critical review: *H.K.P., S.Ü.*; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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