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Research Article [Araştırma Makalesi]

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Cisplatin delivery from nickel supported Al_2O_3 powders: characterization with swelling and mutagenity tests

[Nikel destekli Al_2O_3 partiküllerinden sisplatin salınımı: Şişme ve mutajenite testleri ile karakterizasyonu]

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ABSTRACT

Objective: In this study cisplatin release profile of Al_2O_3 powders with nickel phase supportion was investigated. Swelling property, mutagenity test and FTIR analysis were achieved for characterization of powders.

Methods: Different formulations of nickel supported Al_2O_3 powders were synthesized for cisplatin release studies. Swelling ratio, Fourier transform infrared spectroscopy spectrum and Ames test were investigated for the characterization of Al_2O_3 and nickel supported Al_2O_3 powders. *In vitro* cisplatin release were studied in physiological saline solution and the amount of released cisplatin was determined with spectrophotometric analysis.

Results: The powders showed a swelling degree between 46-86% and swelling ratio increased with increasing Ni phase. This behaviour has been explained on the basis of the softer character of nickel phase. And the results of Ames test indicate that all powder formulations are non-mutagenic. The release of cisplatin was studied as a function of time and all formulations showed non-Fickian diffusion with *n* value ranged between 0.55 and 0.69.

Conclusion: The nickel supported Al_2O_3 formulations designed in this study, shows great promise as a cisplatin release material due to the favorable characteristics identified in these studies.

Keywords: Al_2O_3 powders, nickel, cisplatin release, surface polarity

Conflict of interest: There is no conflict of interest between autors.

ÖZET

Amaç: Bu çalışmada nikel fazı ile desteklenmiş Al_2O_3 partiküllerinin sisplatin salınım profili incelendi. Partiküllerin karakterizasyonu şişme özelliği, mutajenite testi ve FTIR analizi ile gerçekleştirildi.

Yöntem: Sisplatin salınım çalışmaları için farklı formülasyonlarda Al_2O_3 partikülleri sentezlendi. Al_2O_3 partikülleri ve nikel destekli Al_2O_3 partiküllerin karakterizasyonu şişme oranı, Fourier transform infrared spectroscopy spektrumu ve Ames testi ile belirlendi. *In vitro* sisplatin salınımı fizyolojik tuz çözeltisi içerisinde gerçekleştirildi ve salınan sisplatin miktarı spektrofotometrik yöntemle belirlendi.

Bulgular: Sonuçlardan partiküllerin %46-86 aralığında şişme davranışı sergilediği ve partikül yapısında nikel oranının artması ile şişme oranının da arttığı belirlendi. Bu sonuç nikel fazının yumuşak karakteri ile açıklanabilir. Ames testi sonuçları ile tüm formülasyonlardaki Al_2O_3 partiküllerinin mutajenik olmadığı belirlendi. Zamana bağlı olarak incelenen sisplatin salınımında, tüm formülasyonlarda n değeri 0.55-0.69 aralığında bulundu ve Fickian olmayan salınım profili gözlendi.

Sonuç: Bu çalışmada geliştirilen nikel destekli Al_2O_3 partiküllerinin olumlu özellikleri sayesinde sisplatin salınım materyali olarak umut verici olduğu belirlendi.

Anahtar Kelimeler: Al_2O_3 partikülleri, nikel, sisplatin salınımı, yüzey polaritesi

Çıkar çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadı

Introduction

Cisplatin (cis-dichlorodiammineplatinum (II)) is commonly used to treat various types of cancers, including some carcinomas, lymphoma and germ cell tumors [1,2]. After cellular uptake, cisplatin binds to DNA and causes crosslinking of DNA which triggers apoptosis [3]. Chronic cisplatin usage results in cellular cisplatin accumulation which induces cisplatin-resistant disease [4,5]. To break the resistance, the dosing of cisplatin might be adjusted to therapeutic levels. A promising strategy to standarized the therapeutic level of cisplatin is the use of controlled release system that releases the drug at a constant rate continuously. A range of support materials have been employed to control the release of cisplatin such as poly-d, l-lactic acid (CDDP-PLA) [6], hyperbranched polymers [7], gelatin microspheres [8], ethylcellulose-walled microcapsules [9].

Recent researchs in controlled release systems have focused on the development of new biomaterials with high biocompatibility properties. In this study we developed a new nickel supported Al_2O_3 powder and examined in controlled release of cisplatin. Composite Al_2O_3 powders were used as support material in different drug release systems such as ibuprofen [10], deoxyadenosine [11] and tacrolimus [12]. But Al_2O_3 formulation has not been tested yet in cisplatin release.

Al_2O_3 powders are widely used in different industries because of its high strength, good chemical stability, good oxidation and corrosion resistance although high hardness properties [13]. Many approaches have been made to improve the mechanical properties of Al_2O_3 by strengthening the structure with different metal particles [14]. Nickel ductile metal has a potential to improve the hardness properties of Al2O3 because the soft character of Ni phase [15]. In this connection, incorporation of Nickel phase to Al_2O_3 structure was achieved and the changes in characters of Al_2O_3 were investigated with FTIR spectrum, swelling and mutagenity analysis. In continuation, cisplatin release profiles of nickel supported Al_2O_3 powders as a function of time were also analyzed. We believe that this is the first study to examine the drug release profile of nickel supported Al_2O_3 powders.

Material and Methods

Preparation of Nickel supported Al_2O_3 Powders

An aqueous solution containing 0.1M Al^{+3} ($Al_2(SO_4)_3 \cdot 18H_2O$, Merck) and excess urea (Sigma Aldrich) was boiled for 4 h to obtain an alumina precursor precipitate. The precursor precipitate was separated and heated at 1000°C for 4 h. Nickel ($NiBr_2$, Merck) was used as a reinforcing material due to its high strength and toughness [13]. The nickel corporation to powder samples were achieved by blending Al_2O_3, Ni and PVA at the same time using a magnetic stirrer for 4h. 2 wt%polyvinyl alcohol (PVA) was used as a binder for powder compaction. The powder compositions were varied according to nickel additions as 1.0, 3.0, 5.0 and 7.0 wt%and the compositions were called as Ni-Al_2O_3-01, Ni-Al_2O_3-03, Ni-Al_2O_3-05, Ni-Al_2O_3-07 according to Ni composition, respectively. For comparison, Al_2O_3 powder without Ni addition was also prepared by the same process and called as Al_2O_3. Drug loading was achieved by adsorption process. At first, cisplatin (Ebewe Pharma, Unterach, Austria, 50mg/100 ml) was dissolved in water–dimethyl sulfoxide solution (10:1, v/v) [16]. All compositions of Al_2O_3 powders were suspended in cisplatin solution and stirred for 12 h at room temperature in the dark. The suspension was centrifuged at 8000x*g* for 15 min, washed with deionized and the pellet was stored for release studies. Residual cisplatin in the supernatant was analyzed by a modified colorimetric o-phenylenediamine method [17] and the loading efficiency of cisplatin was calculated by Equation 1.

$$E_L\,(\%) = = \frac{(Ctotal - Csupernatant)}{Ctotal} \times 100 \qquad (1)$$

where, E_L is the loading efficiency of cisplatin, C_{total} is the total cisplatin in the loading solution, and Csupernatant is the amount of cisplatin in the supernatant.

Swelling properties

All composition of powders (0.1g) were carefully transferred into a volumetric cylinder and the height of powders was measured (W_0). 50 ml of physiologic buffer was added to the volumetric cylinder. The cylinder was placed in a waterbath (37°C) for 4 h. The increase in the height was measured periodically at certain intervals (W_S). The swelling ratio of powders was calculated by using Equation 2.

$$\text{Swelling ratio: } = = \frac{(W_S / W_0)}{W_0} \times 100 \qquad (2)$$

W_0 and W_S are the heights of powders before and after uptake of water, respectively.

Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectra of the Al_2O_3, Ni-Al_2O_3-07 and cisplatin loaded Ni-Al_2O_3-07 powders were obtained by using Perkin Elmer Paragon 1000 (CT, USA). For this aim, the samples were mixed with KBr and pressed into a tablet form. The FT-IR spectrum was then recorded.

Mutagenity test

Salmonella mutagenicity tests were performed using the standard plate incorporation method with the TA100 strain of *Salmonella typhimurium,* which is capable of detecting base pair substitution–type mutagenicity [18]. 5 ml bacterial culture (12 h) and 0-1000 µg related powder formulation were incubated at 37°C for 1 h in

a rotary shaker. For test of with S9 activation, 0.5 mL S9 mixture, 5 ml bacterial culture (12 h) and 0- 1000 µg related powder formulation were incubated at 37°C for 1 h. After incubation, the contents were poured onto minimal glucose agar plates and the plates were incubated for 48 h at 37°C. The revertant colonies on each plate were counted after 48 h of incubation and the number of revertant colonies in each sample was recorded as the mean value from five plates. Sodium azide was used as positive control.

In vitro release studies

Cisplatin release studies were carried out in a continuous release system consisted of a column with a length of 17 cm and a diameter of 0.9 cm. The cisplatin-loaded carriers (1.9g) were placed in the release cell and the physiological buffer was introduced into the release cell at a flow-rate of 0.1 ml/min using a peristaltic pump. At defined time intervals, samples were collected and assayed for cisplatin release. The amount of released cisplatin was determined spectrophotometrically using o-phenylenediamine method [17] mentioned as above.

The Fickian and non-Fickian absorption of water by powders was determined by using Equation 3 which is often used to describe drug release behavior from polymeric systems.

$$\frac{M_t}{M_\infty} = Kt^n \qquad (3)$$

Where Mt is the amount of cisplatin released at time t, M∞ is the total amount of cisplatin released and K is a constant including structural characteristics of the carrier system and the drug, and n is a constant which relates to the transport mechanism. On taking natural log of Equation 3:

$$\ln \frac{M_t}{M_\infty} = \ln k + n \ln t \qquad (4)$$

the values of n and k were calculated from the slope and intercept of the plot of In Mt/M∞ against In t, respectively.

Results

The cisplatin loading capacities of Ni-Al2O3-07, Ni-Al2O3-05, Ni-Al2O3-03 and Ni-Al2O3-01 were found as 56.6±1.9, 61.5±2.1, 75.0±2.5 and 87.2±1.1%, respectively. The maxiumun drug loading capacity was obtained with Ni-Al2O3-01 powder formulation.

Swelling ratio

Swelling ratios of Al2O3, Ni-Al2O3-07, Ni- Al2O3-05, Ni-Al2O3-03 and Ni-Al2O3-01 compositions were given in Figure 1. The results indicated that it is possible to produce composite materials with different swelling ratios by varying the Ni:Al2O3 rates. Swelling ratio was increased with increasing the nickel phase in composite structure. Maximum swelling ratio was obtained with Ni-Al2O3-07 as 86% while Al2O3 gives minimum swelling ratio as 46%.

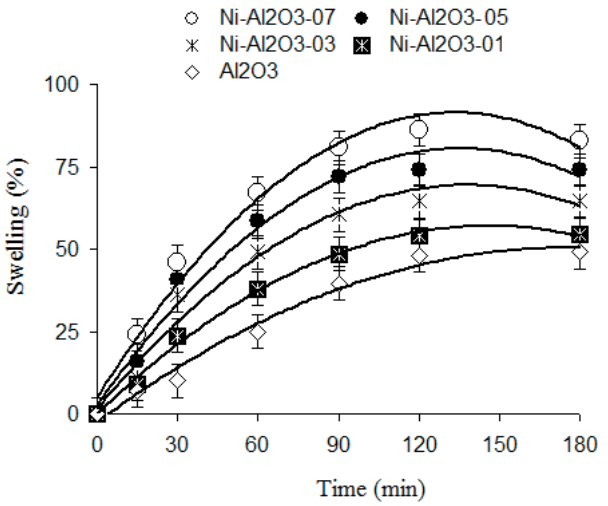

Figure 1. Swelling degrees of powders over a period of 180 min with a temperature of 37°C.

Infrared spectrum

Figure 2 shows the infrared spectrum of the Al2O3, Ni-Al2O3-01 and cisplatin loaded Ni-Al2O3-01 powders which depicts maximum loading. In all spectrum, the peak observed at around 619cm−1 corresponds to the vibration of Al–O bond [19]. In Ni-Al2O3-01 spectrum an additional strong peaks at 445 cm−1 and 490 cm−1 were undoubtedly assigned to Ni–O stretching [20,21]. These results clearly suggest that Ni incorporation successfully achieved by mixing nickel and aluminum phase. As expected in the spectrum of cisplatin loaded Ni-Al2O3-01 powders, characteristic amine stretching peaks of cisplatin were observed at around 3400–3200 cm_1. And a symmetric amine bending of cisplatin was observed at around 1620–1500 cm_1.

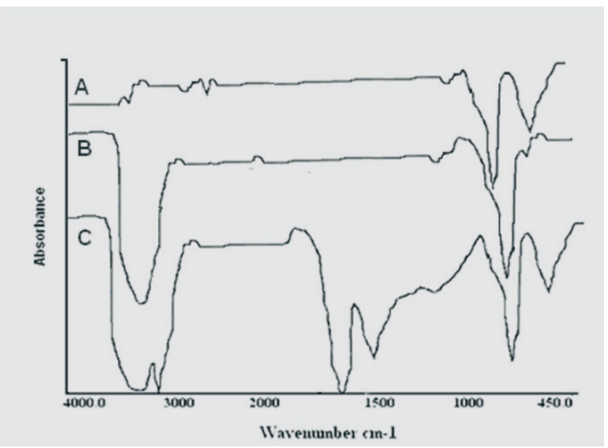

Figure 2. Infrared spectrum of a) Ni- Al2O3-01, b) Al2O3, c) cisplatin loaded Ni-Al2O3-01 powders

Mutagenity test

The mutagenity of nickel supported Al2O3 formulations was tested by using Ames test. The results were consi-

dered positive if the tested sample produced a response which was at least twice as high as the one found with the negative control [18]. An increase in the number of revertent colonies were seen for the positive control, indicating that the test system responded appropriately. No significant increase in the number of reverse mutants were found in treatment groups compared to the positive control groups (Table 1). With S9 activation, the mutagenicity of Al2O3 formulations was negative at all the tested concentrations.

Release profile

Cisplatin release from nickel supported Al2O3 powders has been studied as a function of time. The data obtained from experimental studies show that the different composition of carriers are significantly affected the release behaviour of cisplatin. This phenomen is supported by Figure 3 which depicts the release profiles of cisplatin from Al2O3 powder formulations. The effect of aluminum/nickel phase ratio on the release profile of cisplatin was also investigated. The release percents of Ni- Al2O3-07, Ni- Al2O3-05, Ni- Al2O3-03, Ni- Al2O3-01 and Al2O3 were found as 74.6, 66.1, 60.4, 56.3 and 52.1%, respectively.

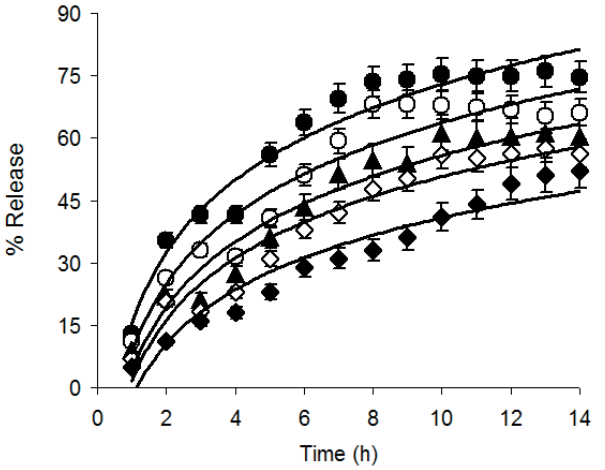

Figure 3. Cisplatin release as a fuction of time (mean ±S.D., n=3); ● Ni- Al2O3-07; ○Ni- Al2O3-05; ▲ Ni- Al2O3-03; □ Ni- Al2O3-01 ; ■ Al2O3

Discussion

In this study we identified the ideal characteristics of the nickel supported Al2O3 powders for cisplatin release. The composite morphology exhibited different changes with nickel introduction to the composite structure.

Table 1. Mutagenic activity of nickel supported Al_2O_3 formulations against T100

Formulation	Dose µg/plate	Revertant number (mean±SD)	
		-S9	+S9
Al_2O_3	0	325±21	356±19
	10	218±18	222±16
	100	205±12	215±13
	1000	211±16	210±11
Ni-Al_2O_3-01	0	325±21	356±19
	10	256±11	261±21
	100	220±13	235±9
	1000	201±17	212±11
Ni-Al_2O_3-03	0	325±21	356±19
	10	261±10	275±8
	100	252±9	263±11
	1000	223±13	247±18
Ni-Al_2O_3-05	0	325±21	356±19
	10	259±8	266±13
	100	276±16	256±9
	1000	253±11	249±11
Ni-Al_2O_3-07	0	325±21	356±19
	10	275±19	269±8
	100	244±22	255±11
	1000	230±9	250±12

Positive control: sodium azide 789 ± 18 colonies

The swelling ratios of Ni- Al2O3-07, Ni- Al2O3-05, Ni-Al2O3-03, Ni- Al2O3-01 and Al2O3 were observed as 82.8, 74, 64.7, 54.4 and 49%, respectively. The increase in swelling ratios of composites with increasing nickel incorporation is mainly due to the softer character of the nickel phase [22]. Rise in the nickel phase of the composites results a decrease in hardness and powder become softer. Similiar results were obtained by Chou and Tuan [23] and Seleman et al. [22]. Likely, Theerabornkul and Kangwantrakool [24] reported that the hardness of Al2O3 sample was decreased with higher amount of Ni and the highest hardness was obtained from Al2O3 sample without Ni addition. In other words, hardness and a swelling ratio have close relations. Likely, Yagi et al. [25] reported that swelling ratios of materials decrease with rising the hardness character.

The developed products for pharmaceuticals and medical applications need a detailed investigation of safety and efficacy to human health. Ames test is a biological assay to assess the mutagenic potential of chemical compounds. The mutagenity of nickel supported Al2O3 formulations was tested by using Ames test. All formulation of powders were found to be non-mutagenic to tested strain. The strain exposed to different concentrations of powder formulations did not showed two-fold or greater increase in the number of revertants compared to the positive control. However, the number of reverse mutant of S. typhimurium TA100 was decreased in the presence of Al2O3 formulations. This result can be explained by the possible microbial growth inhibition effect of Al2O3. Revertant colony numbers became stronger with S9 addition. These result suggested that some new structures occurred after metabolic reactions that enhanced mutagenic interactions.

In vitro examination of blood compatibility of materials can predict the immediate undesirable interactions of materials with various blood components. The biocompatibility of alumina powders has been tested by many researchers. Yalçın et al. [26] studied the biocompatibility of nickel-Al2O3 powders by using plasma protein adsorption test and showed a non-spesific insignificant adsorption of serum proteins. Noiri et al. [27] evaluated the biocompatibility of alumina powders by histopathalogicalstudies in albino rabbits. The results showed no signs of implant rejection or prolapse of the implanted piece. Kanematsu [28] tested alumina composites in L cell line cultures and displayed same colony formation.

The release of cisplatin from nickel supported Al2O3 formulations has been studied as a function of time. In the release profile, cisplatin release was increased with increasing the Ni incorporation. This result can be explained by the increase of swelling degree and diffusion of drug to solvent [20]. Also a slight burst release was observed in the first few hours of release profile. This sudden release may be due to rapid swelling of powders in same period. Similar observations have also been reported elsewhere. Chandy [29] reported that the amount of cisplatin release from poly(lactic acid)–poly(caprolactone) blends was much higher initially (20–30%), and followed by a constant slow-release profile for a 30-day period of study. The n values for all powder formulations were determined in a range of 0.55 and 0.69. In a spherical shaped powders the value of $n \leq 0.43$ indicates Fickian release; $0.45 < n < 0.85$ indicates non-Fickian (anomalous) release [30]. For systems exhibiting anomalous release, the dominant mechanism for cisplatin transport is due to matrix relaxation. In swelling process two underlying molecular way occured. These are the penetration of the solvent molecules into the powder and relaxation of the powder structure [31]. So the matrix relation responsible for cisplatin transport highly related with swelling mechanism. And also a value up to 7.21 was obtained for k values in our study. A higher value of k may suggest burst drug release from the carrier and also the n values of all formulations are within the limits of the non-Fickian transport mechanism. From all these results it is clear that the non-Fickian release mechanism takes place in all formulations meaning that drug release couples diffusion with polymer matrix relaxation and may indicate that drug release is controlled by more than one process.

So many studies of cisplatin release from different polymeric films have been reported. Czarnobaj et al. [32] examined the effect of PEG on cisplatin release profile from silica xerogels and reported that the release of cisplatin from the matrices grows with the increase of PEG volume in xerogel (up to 74–97% within 7 days). Ohta et al. [33] studied the cisplatin release from drug-conjugated gelatin microspheres reported a release rate of 12.4% after 24 h. In another study, Fang-an et al. [34] used a biodegradable polymer as cisplatin delivery device and noted that the polymer released 80% of the loaded cisplatin in vivo over a 7-day period. Hecquet et al. [35] reached 80% release rate of cisplatin with ethylcellulose-walled microcapsules in 24 h. Comparison of these results shows that the cisplatin release rate and loading efficiency of Ni-Al2O3-01-07 powders in our study is in agreement the datas reported in literature. For a prolonged release period slow drug release should be applied. A desired slow release rate can be attained when the structural characteristics of composites (alumina:nickel phase rates) and the loading capacities are varied. After drug release biomaterials exposed to some changes in living media. Ni-Al2O3 powders are able to sustain by some mechanisms in vivo such as extraction of aluminum from the oxide structure or the aging phenomena with reduction in some mechanical properties.

Conclusion

According to the results, it can be concluded that nickel supported Al2O3 formulations are effective material which can be used in several release applications. By varying the powder composition with the suitable characteristic properties and slowest release rate in vitro, we hope to obtain the slowest release with desired level in vivo at same conditions. And also the non-mutagenic na-

ture of powders is also a favourable factor for its possible use in medical fields.

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