

THE INFLUENCE OF THE FORMULATION FACTORS ON PHYSICO-CHEMICAL PROPERTIES OF DEXTRAN ASSOCIATED GANTREZ[®] AN NANOPARTICLES

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Abstract

Gantrez[®] AN (a copolymer between methyl vinyl ether and maleic anhydride) nanoparticles were prepared by the solvent displacement method, and different dextrans with different molecular weight were associated to these nanoparticles in order to modify their surface characteristics. The resulting nanoparticles were characterized in terms of size, zeta potential, amount of dextran associated and dextran association efficiency. Using multifactorial analysis, we have studied the influence of formulation factors on nanoparticles' characteristics mentioned above. The formulation factors studied were the following: the type of dextran used, the preparation method, the incubation time of dextran with the polymer and the quantity of dextran used. The molecular weight of dextran used was the major factor that influenced all nanoparticle properties evaluated, while the other factors studied had limited influence on nanoparticles' characteristics.

Rezumat

S-au preparat nanoparticule din Gantrez[®] AN (copolimer al metilvinil eterului cu anhidrida maleică) prin metoda deplasării solventului. S-a asociat dextran cu mase moleculare diferite acestor nanoparticule pentru modificarea proprietăților lor de suprafață. Nanoparticulele obținute au fost caracterizate din punct de vedere al mărimii, potențialului zeta, cantității de dextran asociate și a eficienței asocierii. Utilizând analiza multifactorială, am studiat efectul factorilor de formulare asupra caracteristicilor nanoparticulelor menționate anterior. Factorii de formulare studiați au fost tipul de dextran utilizat, metoda de preparare, timpul de incubare al polimerului cu dextranul și cantitatea de dextran utilizată. Masa moleculară a dextranului a fost cel mai important factor care a influențat toate proprietățile nanoparticulelor care au fost evaluate, în timp ce ceilalți factori de formulare au avut o influență limitată asupra caracteristicilor nanoparticulelor.

Key words: Gantrez[®] AN; dextran; nanoparticles; multivariate analysis.

Introduction

Polymeric nanoparticles are currently being investigated as drug delivery systems. These systems have several advantages compared with traditional dosage forms such as their ability to deliver the drug to a target site and to minimize its side effects [3]. Nanoparticles' fate in the biological environment is influenced by their physical properties such as size, polydispersity, zeta potential and surface characteristics [7,8].

In order to achieve long circulation of the drugs in the body, drug targeting to the site of action or bioadhesive properties, surface modification of polymeric nanoparticles is frequently used. The material used to prepare the nanoparticles has to be able to control the release of the loaded drug and to have functional groups that permit the modification or decoration of their surface [4]. Gantrez AN, a copolymer between methyl vinyl ether and maleic anhydride (PVM/MA), has been proposed as a material suitable to obtain nanoparticles with the above mentioned characteristics, especially bioadhesive nanoparticles [1,2], the development of bioadhesive dosage forms being currently of interest for both conventional and new dosage forms [6].

Increasing the bioavailability of drugs using oligo- and polysaccharides is currently of great interest. Among oligosaccharides, cyclodextrins are the most used [9,10]. Dextran is a polysaccharide consisting of glucose molecules coupled into long chains through 1,6- and 1,3- glucosidic linkage. Dextrans are widely used in clinic as blood expanders and currently they are being investigated as carrier system for a variety of therapeutic agents [5].

The objective of our work was to associate dextran to PVM/MA nanoparticles and to study the influence of some formulation factors on nanoparticles' characteristics, using multifactorial analysis.

Materials and methods

Chemicals

Gantrez[®] AN 119 [poly (methyl vinyl ether-co-maleic anhydride); MW 200,000] was a gift from ISP (Barcelona, Spain). Dextran MW 70000 (Dex70) and dextran MW 40000 (Dex40) were purchased from Sigma–Aldrich Chemie (Germany). All other chemicals used were of reagent grade.

Preparation of Gantrez[®] AN nanoparticles

Poly (methyl vinyl ether-co-maleic anhydride) nanoparticles were prepared by a solvent displacement method [1]. For dextran association, two

different methods were assayed. Method A consisted in the incubation of Gantrez with either Dex 40 or Dex 70 in the acetonic phase, prior to nanoparticle formation by the addition of an ethanol-water mixture (1:1). The organic phase was then eliminated under reduced pressure (Buchi R-144, Switzerland).

Method B consisted in the preparation of unloaded Gantrez nanoparticles, which were subsequently incubated with dextran aqueous solutions, for different periods of time, at room temperature.

In both cases, the nanoparticles were purified twice by centrifugation at 20000 rpm, for 20 minutes (Sigma 3K30, Germany). The supernatants resulted in the purification steps were recovered in order to quantify the unloaded dextran by HPLC.

Finally, the nanoparticles were lyophilized using sucrose (5%) as cryoprotector. The control nanoparticles (NP) were prepared as described above in the absence of dextran.

Nanoparticles' characterization

The particle size and zeta-potential were determined by photon correlation spectroscopy and electrophoretic laser Doppler anemometry using a Zetamaster analyzer (Malvern Instruments, UK). For particle size analysis, samples were dispersed in ultra pure water and measurements were carried out at 25°C with a scattering angle of 90°.

The yield of nanoparticle preparation process was calculated by gravimetry. For that purpose, freshly-prepared nanoparticles were freeze-dried in the absence of cryoprotector and the yield was calculated as the difference between the initial amount of the polymer used to prepare nanoparticles and the weight of the freeze-dried product obtained.

The amount of dextran associated to the particles was determined by quantification of unloaded dextran in the supernatants obtained during the purification steps using a HPLC with evaporative light scattering detection (ELSD) method. The apparatus used for the HPLC analysis was an Agilent model 1100 series LC (Waldbronn, Germany) coupled with an evaporative light scattering detector (Alltech, Illinois, USA). The separation was performed in the following conditions: temperature 40°C, PL Aquagel-OH column (300nm×7,5mm), mobile phase consisting of a mixture of methanol and water in a gradient elution at a flow-rate of 1 mL/min. The ELSD conditions were set as follows: drift tube temperature was maintained at 110°C and nitrogen flow was set at 3L/min.

The amount of dextran associated to the nanoparticles was calculated as the difference between the initial amount of dextran added and

the amount of dextran measured by HPLC in the supernatants, after the centrifugation steps.

The association efficiency (%) was expressed as the percentage of dextran associated to nanoparticles with respect to the initial amount of dextran added in the preparation process.

Experimental design

For nanoparticle preparation, some formulation factors were studied in order to optimize dextran association to Gantrez[®] AN nanoparticles. These factors (independent variables) and their levels of variation are presented in Table I. The effect of independent variables was investigated on NP size, zeta potential, quantity of dextran associated ($\mu\text{g}/\text{mg}$ nanoparticle) and on association efficiency (%) (Table II). Table III presents the matrix of the performed experiments.

Multivariate analysis

In order to determine a statistical correlation between the formulation factors studied and the observed responses, multivariate analysis and statistical parameters calculations were performed with the statistical module from Modde 6 software (Umetrics, Sweden), using Partial Least Squares method.

Table I
Independent variables and their level of variation

Formulation factors	Symbols	Level				
Dextran molecular weight (Mw)	X ₁	40000		70000		
Preparation method	X ₂	A		B		
Incubation time (min)	X ₃	5	15	30	60	90
Dextran amount (mg)	X ₄	10		20		35

Table II
Dependent variables (responses)

Exp. No.	Responses	Symbols
1	NP size (nm)	Y ₁
2	Zeta potential (mV)	Y ₂
3	Association efficiency (%)	Y ₃
4	Dextran associated ($\mu\text{g}/\text{mg}$)	Y ₄

Table III
Matrix of experimental design

Exp Name	X ₁	X ₂	X ₃	X ₄	Exp Name	X ₁	X ₂	X ₃	X ₄
N1	40000	A	5	10	N21	70000	A	5	35
N2	40000	A	5	20	N22	70000	A	15	10
N3	40000	A	5	35	N23	70000	A	15	20
N4	40000	A	15	10	N24	70000	A	15	35
N5	40000	A	15	20	N25	70000	A	30	10
N6	40000	A	15	35	N26	70000	A	30	20
N7	40000	A	30	10	N27	70000	A	30	35
N8	40000	A	30	20	N28	70000	B	30	10
N9	40000	A	30	35	N29	70000	B	30	20
N10	40000	B	30	10	N30	70000	B	30	35
N11	40000	B	30	20	N31	70000	B	60	10
N12	40000	B	30	35	N32	70000	B	60	20
N13	40000	B	60	10	N33	70000	B	60	35
N14	40000	B	60	20	N34	70000	B	90	10
N15	40000	B	60	35	N35	70000	B	90	20
N16	40000	B	90	10	N36	70000	B	90	35
N17	40000	B	90	20	N37	70000	B	5	20
N18	40000	B	90	35	N38	70000	B	5	35
N19	70000	A	5	10	N39	70000	B	5	10
N20	70000	A	5	20	N40	70000	A	5	10

X₁ - Dextran Mw, X₂ – Preparation method, X₃ – Incubation time (min), X₄ – Dextran amount (mg)

Results and discussion

Nanoparticles' preparation and characterization

Gantrez[®] AN nanoparticles can be obtained by a simple solvent displacement method. The yield of the preparation process had good values, between 75% and 98%, for all the formulations. Moreover, some simple modifications of this method permit the association of different compounds such as dextrans to the polymeric nanoparticles, the objective of this association being the modification of their *in vivo* behavior, mainly their bioadhesive interactions with the gastrointestinal tract. The nanoparticle characteristics (NP size, zeta potential, association efficiency and dextran associated) are shown in Table IV.

Table IV
Matrix of responses

Exp Name	Y ₁	Y ₂	Y ₃	Y ₄	Exp Name	Y ₁	Y ₂	Y ₃	Y ₄
N1	126.7	26.7	28.8	28.8	N21	195.3	50.4	39.5	11.3
N2	135.7	30.4	22.0	11.0	N22	175.3	50.8	13.0	13.0
N3	137.7	27.1	39.4	11.3	N23	197.7	52.4	20.9	10.5
N4	137.7	23.3	19.5	19.5	N24	191.0	49.9	33.1	9.5
N5	132.0	31.7	34.9	17.5	N25	245.3	53.7	13.0	13.0
N6	128.0	31.7	49.3	19.8	N26	193.7	58.2	23.8	11.9
N7	138.3	29.0	22.2	22.2	N27	164.7	55.8	21.5	6.1
N8	140.0	33.8	34.9	17.4	N28	198.0	58.5	15.2	16.0
N9	148.3	33.1	26.4	7.6	N29	197.0	60.7	32.0	17.0
N10	198.3	31.8	19.3	19.3	N30	205.3	58.8	44.4	14.6
N11	203.3	32.8	62.8	31.4	N31	182.3	64.2	12.0	12.0
N12	201.3	31.8	75.2	21.5	N32	193.3	63.1	33.0	16.5
N13	193.7	39.7	42.0	42.0	N33	200.7	60.6	40.0	11.4
N14	194.0	29.8	48.5	24.3	N34	190.7	68.3	17.0	17.0
N15	184.3	31.1	77.4	22.1	N35	203.0	60.1	34.0	17.0
N16	230.0	20.7	20.7	20.7	N36	193.0	56.3	56.0	16.0
N17	223.0	32.6	35.0	17.5	N37	188.7	58.6	37.4	18.7
N18	214.3	14.1	17.0	4.9	N38	177.0	60.7	78.4	22.4
N19	171.3	54.6	15.3	15.3	N39	194.0	57.8	8.5	8.5
N20	178.7	59.1	15.5	7.8	N40	165.7	56.8	6.5	9.5

Y₁ - NP size; Y₂ - Zeta potential; Y₃ - Association efficiency; Y₄ - Dextran associated

The results obtained after multivariate analysis and the values of the statistical parameters indicated a good fit for NP size, zeta potential and quantity of dextran associated, while for the association efficiency the fit was only satisfactory.

The influence of formulation factors on nanoparticle properties

The influence of the formulation factors on nanoparticle size, zeta potential, dextran association efficiency and on the amount of dextran associated is presented in Figure 2.

The size of nanoparticles (Y₁) was influenced by the type of dextran used (X₁), preparation method (X₂) and the incubation time of dextran with the polymer (X₃) (Fig. 2, A). When using Dex 70, the resulted nanoparticles had an average size greater than in the case of Dex40. We also can observe that in the case of incubating the preformed nanoparticles with dextran aqueous solution (Method B) the resulted nanoparticles had greater

average size than that of nanoparticles obtained by Method A. This increasing of nanoparticle size for Method B can be due to a deposition of dextran at the surface of the preformed nanoparticles.

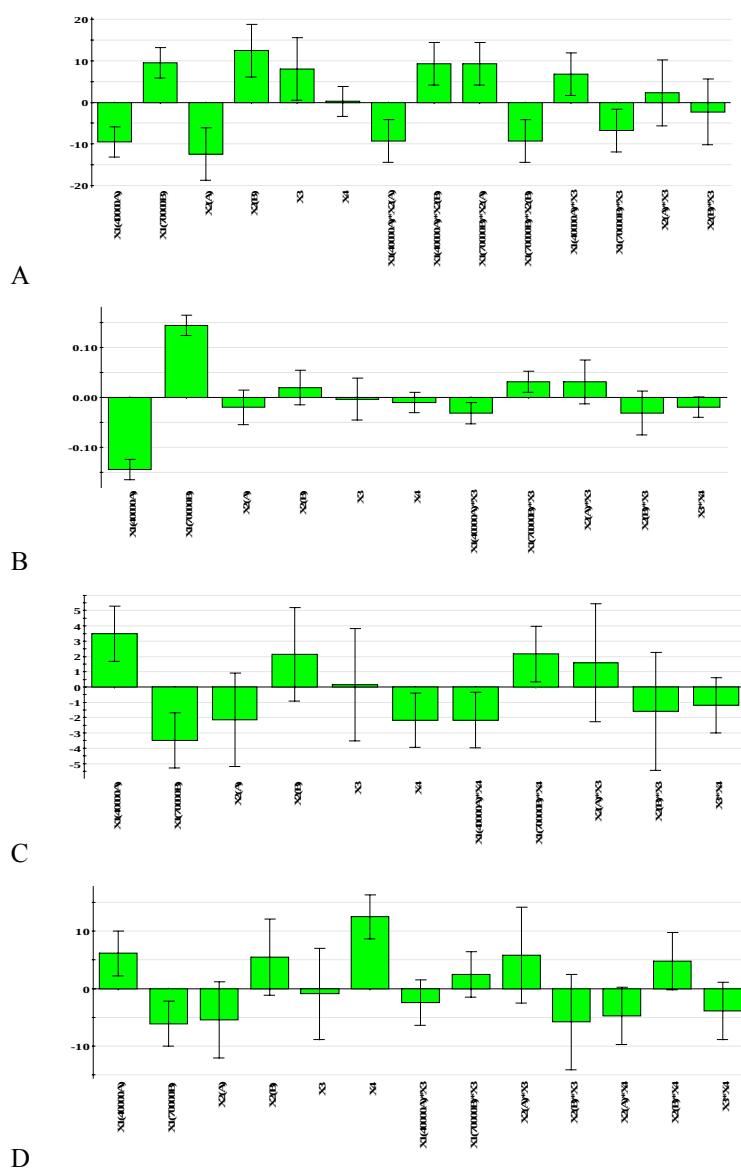


Figure 2

The influence of the formulation factors on: nanoparticle size (A), zeta potential (B), encapsulation efficiency (C) and the amount of dextran associated (D)

However, if we look at the interactions between the two factors X_1 and X_2 (dextran molecular weight and preparation method) the increase of nanoparticle size in Method B was significant only when using Dex 40.

The values of zeta potential (Y_2) were influenced by the type of dextran used: zeta potential increased when Dex 70 was used. Also, in the case of Dex 70 these values increased with the increasing of the incubation time, while for Dex 40 the zeta potential values were smaller when the incubation time increased. Zeta potential is a parameter that gives us information about the charge of nanoparticles' surface. Nanoparticles made of Gantrez have negative charge due to the carboxyl groups resulted by partial hydrolysis of polymer in water [9]. The association of dextran to Gantrez nanoparticles leads to a reduction in zeta potential values, because dextran is a polymer without electrical charge. So we can conclude that in the case of Dex 40 a better deposition at the nanoparticle surface was obtained compared to Dex 70.

Dextran association efficiency (Y_3) was influenced by the type and the amount of dextran used in the preparation process. Higher association efficiency was obtained in the case of Dex 40. The association efficiency increased when lower dextran amounts were used in the preparation process. This observation can be due to a saturation phenomenon that could appear with increasing the quantity of dextran added in the preparation process.

The quantity of dextran associated to the nanoparticles (Y_4) was influenced by the molecular weight of dextran and by the quantity of dextran used. Dextran loading increased when higher amounts of dextran were used in the fabrication process. Also, dextran loading was better for dextran 40 than for dextran 70. The incubation time had a positive influence on dextran loading for both dextrans, in the case of Method A and when using 10 mg dextran in the preparation process, but had no influence on dextran loading when preparing the nanoparticles by Method B.

Optimum formula determination

The optimization module from Modde 6 software permits the determination of the optimum formula, for each dextran used. For this purpose, we have chosen the highest values for dextran association efficiency, the average nanoparticle size obtained for each dextran and the lowest value for zeta potential values. The optimum formulation for Dex 40 was obtained by Method B, with an incubation time of 90 minutes and using 10 mg dextran, while for Dex 70 the optimum formula was obtained by Method A, with an incubation time of 5 minutes and using 35 mg dextran.

Conclusions

This work presents the possibility to obtain polymeric nanoparticles associated with dextran by the solvent displacement method. The preparation method was modified in order to associate dextran to Gantrez nanoparticles and we have demonstrated the influence of the formulation factors such as the type of dextran, the preparation method, the quantity of dextran used and the incubation time on nanoparticles' properties. The major parameter that influenced the characteristics of the prepared nanoparticles was dextran molecular weight, so we can conclude that the type of dextran used is the determinant factor for the physico-chemical properties of the nanoparticles.

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References

1. Arbós P., Wirth M., Arangoa M.A., Gabor F., Irache J.M. Gantrez AN as a new polymer for the preparation of ligand-nanoparticle conjugates. *J Contr Rel* 2002; 83:321-330.
2. Arbós P., Arangoa M.A., Campanero M.A., Irache J.M. Quantification of the bioadhesive properties of protein-coated PVM/MA nanoparticles. *Int. J. Pharm.* 2002; 242: 129-136.
3. Beck R.C.R., Haas S.E., Guterres S.S. Nanoparticle-coated organic-inorganic microprticles: experimental design and gastrointestinal tolerance evaluation. *Quim. Nova* 2006; 29(5): 990-996.
4. Irache J.M., Huici M., Konecny M., Espuelas S., Campanero M.A., et al. Bioadhesive properties of Gantrez nanoparticles. *Molecules* 2005; 10:126-145.
5. Jung S.W., Jeong Y.I., Kim Y.H., Choi K.C., Kim S.H. Drug release from core-shell type nanoparticles of poly(DL-lactide-co-glicolide)-grafted dextran. *J Microencapsulation* 2005; 22(8): 901-911.
6. Popovici I., Ochiuz L., Popa E.A., Saramet G., Dorneanu V., et al. Swellable matrices with carbopols for benzydamine HCl delivery from bioadhesive oromucosal tablets. *Farmacia* 2008; 56(1): 83-91.
7. Prabu P., Chaudhari A.A., Aryal S., Dharmaraj N., Park S.Y., et al. In vitro evaluation of poly(caporlactone) grafted dextran (PGD) nanoparticles with cancer cell. *J Mater Sci: Mater Med* 2008; 19(5): 2157-2163.
8. Shahani K., Jonnalagadda S., Handa H., Mao G.; Panyam J. Phospholipid Nanoparticles: Process Optimization Using Factorial Design and Atomic Force Microscopy. *J. Biomed. Nanotechnol* 2007; 3(4): 394-400.

9. Rus L., Constantinescu D., Drăgan F., Farcas A., Kacsó I. Inclusion complex of enalapril maleate/ β -cyclodextrin. FT IR, X-ray diffraction, DSC and molecular modeling. *Farmacia* 2007; 55(2): 185-192.
10. Trandafirescu C., Gyéresi A., Kata M., Aigner Z., Szabadai Z. Study of albendazole hydroxypropyl- β -cyclodextrin binary systems. *Farmacia* 2007; 55(5): 580-589.
11. Yoncheva K., Lizarraga E., Irache J.M. Pegylated nanoparticles based on poly(methyl vinyl ether-co-maleic anhydride): preparation and evaluation of their bioadhesive properties. *Europ. J. Pharm. Sci.* 2005; 24: 411-419.

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