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Influence of the acid type on the physical and drug liberation properties of chitosan–gelatin sponges

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Abstract

The influence of acid type used to dissolve chitosan on the resulting sponge physical properties, and their consequent effect on the drug liberation were investigated. Chitosan was dissolved in different acid solutions and chitosan–gelatin sponges were produced by frothing up the polymer solution and then freeze-drying the foam. Prednisolone was used as a model drug. Using tartaric or citric acid resulted in instable, soft, elastic and disintegrating sponges with fast drug release. Elastic but harder sponges from stable foams were obtained when hydrochloric or lactic acid were used. The use of acetic or formic acid enabled the production of stable foams, soft and elastic sponges and a slow drug release. The rate of drug release was decreased by crosslinking the polymers with glutaraldehyde, but only if acetic, formic or acetic acid were used. Therefore, it is possible to manipulate the mechanical properties and the drug liberation rate by using different acids to dissolve chitosan.

Keywords: Chitosan–gelatin-sponge; Acid type; Glutaraldehyde crosslinking; Controlled release; Physical properties

1. Introduction

Porous biomaterials can provide a frame work for the ingrowth of connective tissue. They can absorb wound fluid and promote healing. The porosity and surface characteristics of the three dimensional meshwork of sponges is an essential feature for cell ingrowth. For this reason sponges are used in wound treatment, as hemostatics, after tooth extraction or for implantation (Chvapil and Holusa, 1968; Chvapil, 1977; Lipsky and Lamberton, 1989). There are numerous products (Gelfoam™, Gelastypt™, Sulmycin™ implant, Cutinova™ hydro a.m.o.) on the market.

For implantation biodegradable materials have to be used as taking out the sponge after treatment always causes new damage to the wound. The patients' comfort and an optimum healing process require a soft and elastic material. In addition to the wound treatment purpose sponges may also serve as drug carriers (Matsuda et al., 1992; Oungbho, 1997).

Chitosan is an deacetylated derivative of chitin. It is insoluble in water and common organic solvents but soluble at a pH under 6.5 in most

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acidic media. Recently there was a considerable interest in chitosan as a drug carrier, e.g. in tablets (Adusumilli and Bolton, 1991), microparticles (Chithambara Thanoo et al., 1992; Berthold et al., 1996), films (Kanke et al., 1989), beads (Aral and Akbuga, 1998), gels (Kristl et al., 1993), and as a absorption enhancer for nasal and oral drug delivery (Illum et al., 1994; Lueßen et al., 1996). Chitosan is well known for its nontoxicity, biocompatibility, biodegradability and its ability to improve healing (Muzzarelli et al., 1988; Chandy and Sharma, 1990). Chitosan solutions alone do not foam at all but chitosan can enhance foaming of protein solutions effectively (Poole, 1989). Gelatin is a biodegradable polymer of wide safety and shows, in contact to chitosan, good foam building properties. Chitosan and gelatin are cheap materials so they can enable an economic production of drug carrier systems. Gelatin forms polyionic complexes with chitosan at the suitable pH value (Thacharodi and Panduranga Rao, 1995). These complexes show a slower rate of dissolution than chitosan at the corresponding pH. Therefore a combination of chitosan and gelatin in a sponge will firstly lead to an absorption of the wound fluid and secondly slowly release a wound treating drug (Oungbho and Müller, 1997). The release profile and biodegradability of both polymers can be effectively controlled by glutaraldehyde crosslinking (Tabata and Ikada, 1989; Jameela et al., 1998).

In this study prednisolone was used as a model drug. To produce foams chitosan has to be dissolved in acidic solution. The standard acid used is acetic acid 1% (w/w). Investigations about the dissolution characteristics of chitosan have revealed that the dissolution rate varies according to the acid type used for dissolving chitosan (Filar and Wirick, 1978; Lin and Lin, 1992). With this knowledge the influence of the acid type on the release characteristics and mechanical properties of chitosan–gelatin sponges is examined in this study. In addition to acetic acid, citric, formic, hydrochloric, lactic and tartaric acid were also used, each at the minimum concentration to dissolve the needed amount of chitosan. Investigations were carried out to determine whether the change of acid type can serve as a way to manipulate release characteristics of chitosan–gelatin sponges to fit certain release profiles.

2. Materials and methods

2.1. Materials

Chitosan [molecular weight 410 000, viscosity 14 mPas (1% solution in 1% acetic acid), degree of deacetylation 92%] was purchased from Fish Contract Bremerhaven, Bremerhaven, Germany. Gelatin type B (180 bloom) was obtained from DGF STOESS (Eberbach/Baden, Germany). Glutaraldehyde (50%) was from Fluka (Buchs, Switzerland). Micronized prednisolone was kindly donated by Jenapharm (Jena, Germany). All acids were of reagent grade or higher. Methanol and acetonitrile were of chromatographic grade.

2.2. Production of sponges

For every acid type the minimum concentration to dissolve 1% (w/w) chitosan was determined by dispersing 0.2 g chitosan in 19.8 g of differently concentrated acidic solutions overnight. The lowest concentrated acidic solution that dissolved chitosan was used for sponge production. Solutions of both polymers were prepared separately as follows: 10 g of gelatin (5% (w/w)) were dissolved in demineralized water and the pH was adjusted to 7.4. One g of chitosan (1% (w/w)) was dissolved in diluted acid solution. The chitosan solution was filtered to remove insoluble impurities. Crosslinking was carried out separately for each polymer by adding 1.5 ml and 0.75 ml of a glutaraldehyde solution (5% (w/w)) to the polymer solution, respectively. After adjusting the pH of the gelatin solution to 7.9, the mixtures were shaken overnight at 37°C. Prednisolone was levigated in 1 ml of ethanol, then chitosan and gelatin solutions were added. The mixture was whipped at 500 rpm and 25°C for 15 min to produce a foam. The foam was freeze-dried afterwards. Cubes with an edge length of 1 cm were cut for further investigation.

2.3. Viscosity of the bulk solution

The viscosity of both polymers in 1% (w/w) (chitosan) or 5% (w/w) solution (gelatin) in acid solution or water, respectively, was determined with a rotation viscosimeter (Rheoanalyser, Contraves, Gieres, France) equipped with a cylindrical measurement unit using the DIN 125 vessel. The viscosity was monitored at a shear rate of 500/s.

2.4. Apparent density

After foaming up was performed the apparent density of the foam was determined immediately by weighing a known volume of foam and calculating the quotient of mass and volume. After freeze-drying at least 10 sponge cubes of 1 cm^3 were weighed to determine the apparent density of the dry product.

2.5. Foam stability

A freshly whipped foam sample was filled into a 100 ml beaker and observed for 60 min. The time with no liquid drainage was noted as foam stability time.

2.6. Drug content

A sponge cube was soaked overnight in 50 ml of a mixture of methanol and 0.1 N hydrochloric acid (1:1 (v/v)). The prednisolone concentration was determined by HPLC using a non-polar column (LiChrospher 100 RP18, 5 μm, Merck, Darmstadt, Germany) and a mixture of acetonitrile, methanol and water (22:21:57 (v/v)) as eluent. The HPLC system was equipped with a pump (Gynkotek, Model 300, München, Germany), an auto sampler (HPLC 360, Kontron, Munich, Germany), a multiple wavelength detector (Type SPDGA, Techlab, Frankfurt, Germany) and an integrator (Shimadzu, Kyoto, Japan). The flow rate of the mobile phase was adjusted to 1.0 ml/min. A volume of 15 μl of assay solution was injected and the eluent was detected at a wavelength of 240 nm. The injection was performed in duplicate. The peak of prednisolone was completely separated from the peaks of buffer solution, chitosan and gelatin. Before injection the sample was neutralized with 0.1 N sodium hydroxide solution and diluted with eluent. The measurement was carried out in triplicate.

2.7. Drug release

The dissolution rate of prednisolone was measured at $37 + 0.1$°C in flow-through cells (diameter of 20 mm, Dissotest CE 6, Sotax, Basel, Switzerland) with 100 ml phosphate buffered saline per sponge cube (pH 7.4, 0.011 M, DAB 1998, including 0.1% sodium edetate for stabilization of the drug). The dissolution medium circulated at a flow rate of 0.5 ml/min in a closed system. Samples were taken from each medium reservoir after 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 10 h, filtered through cellulose acetate membrane filters (0.45 μm) and the sample volume was substituted with fresh buffer solution. Dissolution tests were performed for ten hours with no regard to the released amount of the drug. After dilution with eluent the sample was analyzed by HPLC as described before for drug content. The measurement was performed in triplicate.

2.8. Determination of the residual acid content

The sponge was milled with liquid nitrogen to increase the surface and shorten diffusion distances. Approximately 100 mg of the milled sponge (precisely weighted) were dipped in 75 ml bidistilled water for three days. To prevent determination of undissolved material the test tubes were centrifuged at 42 000 rpm for 30 min. After centrifuging the acid amount of 50 ml of the supernatant was potentiometrically titrated with 0.02 N NaOH. Pure polymers were treated in the same manner to obtain their acid content which had to be subtracted from the results above.

2.9. Mechanical properties

Before the determination of the mechanical properties the sponges were stored under controlled humidity (54% r.H.). The elasticity and hardness of the sponges were measured using the TA-XT2 texture analyser (Stable Micro Systems,

Surrey, UK). The instrument compresses the sponge to 40% of its height twice and plots stress force against time. The hardness [N] was calculated as the peak force for the first compression. Elasticity represents the quotient of the force needed for second and first compression. For an ideally elastic sample this quotient would theoretically have a value of 1. In practice, real samples rarely exceed values of 0.95.

3. Results

3.1. Foam production

One proposed application for chitosan is the use in dentistry (Sapelli et al., 1986). Especially at this site of application a neutral taste of the drug carrier is favorable. Hence, different acids than the standard solvent acetic acid were examined for their suitability in sponge production.

The foam-building capacity of chitosan–gelatin mixtures strongly depends on the acid type. Foams which contained 4% tartaric acid or 3% citric acid were not stable (Table 1a). In contrast to this, foams produced with other acids showed a stability time of more than 60 min. After freeze-drying no phase separation was observed. To investigate the possible influence of the acid amount sponges were produced with 0.5, 1, 2 and 4% acetic acid. The amount of acetic acid affected the apparent density and foam stability, as a higher amount of acetic acid (2 and 4%) caused instability (Table 1a). After freeze-drying, sponges produced with 0.5 and 1% acetic acid were more homogenous than sponges with 2 and 4% acetic acid because of the insufficient foam stability of the latter batches.

3.2. Viscosity of the bulk solution

The viscosity of chitosan solutions is a function of the type of acid — and its concentration —

Table 1
Effect of various acid types on the bulk properties (a) (solutions and foams) and sponge properties (b)

Acid type	Ace[a]				HCl[b]	For[c]	Lac[d]	Cit[e]	Tar[f]
(a) *Bulk properties*									
Bulk concentration (%)	0.5	1	2	4	0.26	0.5	1	3	4
pH of the bulk solution	5.21	4.59	4.26	3.85	4.82	4.16	4.61	3.50	2.92
Apparent density (mg/cm^3)	200.1	231.7	244.97	224.1	329.1	186.1	269.7	216.1	258.3
Foam stability time (min)	>60	>60	<10	<5	>60	>60	>60	<20	<20
(b) *Sponge properties*									
Apparent density (mg/cm^3)	9.56	10.55	9.99	10.75	16.31	9.63	14.92	8.18	10.64
Elasticity	0.93	0.93	0.92	0.91	0.93	0.92	0.93	0.91	0.92
Hardness (N)	0.72	1.26	0.75	0.89	2.02	0.48	1.65	0.44	0.65
Dissolution speed (%/h)[g]	6.42	6.64	8.29	5.16	13.47	6.49	16.50	7.41	9.29

[a] Acetic acid.
[b] Hydrochloric acid.
[c] Formic acid.
[d] Lactic acid.
[e] Citric acid.
[f] Tartaric acid.
[g] Slope of dissolution plot in the first 2 h.

Table 2
Dynamic viscosity of chitosan, gelatin and chitosan–gelatin solutions at a shear rate of 500/s

Acid type and concentration	Viscosity of 1% chitosan in acidic solution (mPas)	Viscosity of chitosan–gelatin mixture (mPas)
1% Acetic acid	11.70	21.53
0.5% Formic acid	11.74	17.70
0.26% Hydrochloric acid	10.90	34.10
1% Lactic acid	12.30	26.00
3% Citric acid	10.34	7.54
4% Tartaric acid	10.34	8.67
Gelatine	Viscosity of 5% (w/w) solution in distilled water (mPas)	–
–	8.11	–

used as a solvent (Filar and Wirick, 1978). For this reason, the effect of the acid type on the viscosity of a mixture of chitosan solution and gelatin solution was investigated. It was noted that citric and tartaric acid decreased the viscosity to less than 50% of the acetic acid value (Table 2). The addition of hydrochloric and lactic acid, conversely, increase the viscosity. In case of formic acid a 17% reduction in viscosity in comparison to the acetic acid value was observed. After crosslinking the viscosity of the bulk solutions increased (data not shown).

3.3. Apparent density

The apparent density of foams and sponges varied between the batches when different acid types were used (Table 1a and b). The values of apparent density for foams were generally higher than those for sponges, as in foams about 95% (w/w) of water is incorporated. Hence, monotonical changes in the apparent density were marked for foams and sponges: The addition of tartaric, citric and formic acid lowered the apparent density compared to the standard formulation with 1% acetic acid. In contrast, lactic and hydrochloric acid increased the density.

3.4. Drug release

It is evident from literature that microcapsules containing acetic acid show a more compact structure and exhibit a slower release when compared with other microcapsules made from ascorbic or citric acid (Lin and Lin, 1992). In comparison with the standard sponge produced with 1% acetic acid, the use of 0.5% formic acid leads to a slower release of the drug from uncrosslinked sponges (Fig. 1), whereas citric, tartaric, hydrochloric and lactic acid accelerate the release of prednisolone.

Another possibility to control the drug dissolution from the matrix is the extent of crosslinking as chitosan and gelatin are susceptible to crosslinking with dialdehydes such as glutaraldehyde and such crosslinking effectively controls drug diffusion from the matrix (Tabata and

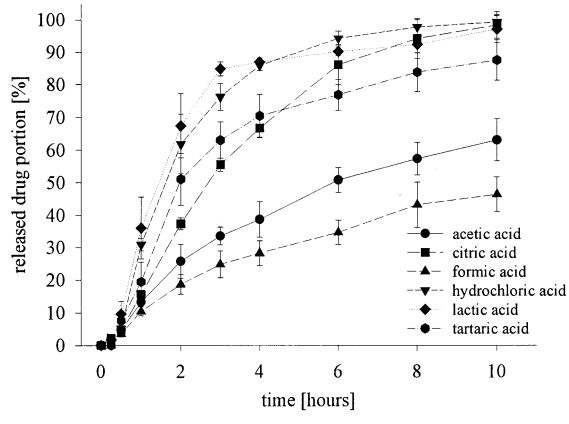

Fig. 1. Dissolution profiles of sponges produced with different acid types: uncrosslinked polymers.

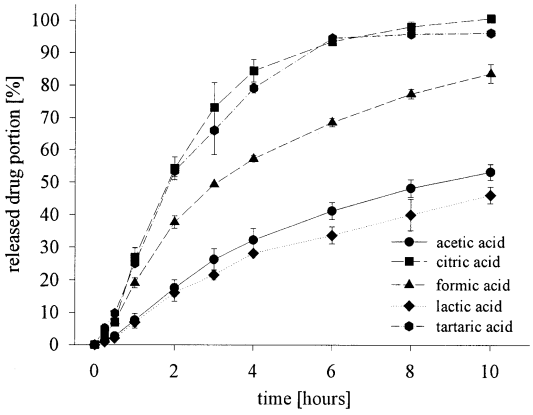

Fig. 2. Dissolution profiles of sponges produced with different acid types: crosslinked polymers.

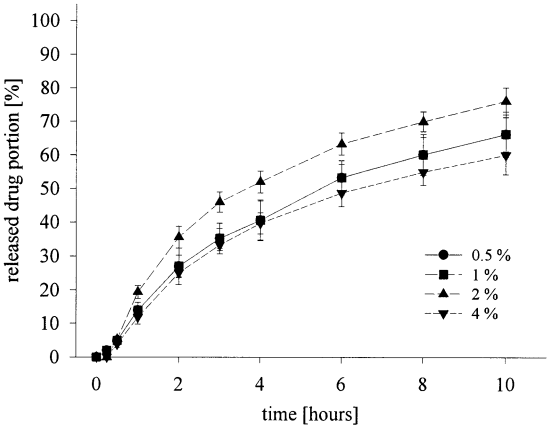

Fig. 3. Dissolution profiles of sponges produced with different amounts of acetic acid.

Ikada, 1989; Jameela et al., 1998). As expected, after crosslinking the release of prednisolone is slowed down when lactic, acetic and formic acid are used in chitosan–gelatin sponges as compared to the uncrosslinked sponges. As tartaric or citric acid are used to dissolve chitosan a negligible change in the release rate was observed by crosslinking (Fig. 2).

With regard to data reported by Lin and Lin (1992), which say that faster drug release rates from microcapsules with higher acetic acid concentration were achieved, the acetic acid concentration was shifted from 0.5 to 4% to significantly change the drug release from sponges (Fig. 3). However, changing the amount of acetic acid does not relevantly influence the release characteristics of chitosan–gelatin sponges.

3.5. Residual acid content

During this study it was taken into consideration if the residual acid content can affect the drug dissolution, as the residual acids in the sponge may serve as solvents for chitosan and, therefore, accelerate the drug release. Hence, the acids in this study were each used in their minimum concentration to dissolve 1% (w/w) chitosan. The acid amount in the sponge after drying process influenced the material, depending on the acid strength and the solving properties for chitosan. Volatile acids are removed to some extent during freeze-drying. Solid acids remain completely in the dry product. Table 3 shows the calculated (supposing no loss of acid) and determined acid contents of the sponges. The results are in good accordance with the calculations. Acetic and formic acid evaporated partially during freeze-drying. The calculated amount of hydrochloric acid was slightly lower than the measured amount. These differences occured due to production or/and measurement imprecision as hydrochloric acid was incorporated in a very low concentration (0.26% (w/w)). Tartaric and citric acid containing solutions formed instable foams, because liquid was drained after 10 and 5 min, respectively. Before freezing phase separation occurs, unsolved particles sedimentated and inside the foam lamellas concentration gradients might appear. For this reason, the measured acid contents for solid acids were lower than the calculated ones.

3.6. Mechanical properties

The mechanical properties of chitosan–gelatin sponges were greatly influenced by the nature of acid. Sponges with an addition of hydrochloric and lactic acid were harder than those with acetic acid (Table 1b), whereas formic, citric and tartaric acids created softer sponges. However, all described sponges were highly elastic (elasticity $<$ 0.9).

4. Discussion

Both the foaming properties of protein solutions and of oppositely charged polymers, are pH dependent (Poole, 1989). The isoelectric point of the gelatin used in this study had a value of 5.1, whereas at pH 7.4 it was negatively charged. Chitosan in acid solution is positively charged. Mixing both solutions resulted in the formation of a polyionic complex. The ideal pH value for complexation of chitosan and gelatin B is between 4.5 and 5.5 (Remunán-López et al., 1996). The complex had a poorer solubility than both of the building polymers. Moreover, a consequent increase of solution viscosity was observed as a result of the formation of the complex (Table 2). The pH value of the polymer mixture before foaming up depended on the acid type (Table 1a), more precisely on the acid concentration and acid strength. Using an acid type which decreased the pH value of chitosan solution, for instance citric acid or tartaric acid, resulted in lower acidic values of the gelatin/chitosan mixture. Consequently, a higher positive or lower negative charge of gelatin would be obtained. Thus, the formation of the gelatin–chitosan complex was impeded. Hence the viscosity of the solution decreased (Table 2) and the whipping can be performed more effortless. Therefore, after freeze-drying sponges with a lower apparent density and different mechanical properties will result when various acid types are used (Table 1b). Due to a larger surface and less diffusional pathways the release from sponges containing citric and tartaric acid was faster. Furthermore, without a complexation of chitosan and gelatin the release of the drug was faster than with complexation. Therefore citric and tartaric acid caused a faster release rate than formic and acetic acid.

The viscosity of the bulk solution influenced hardness of the sponge. Low viscous solutions facilitated the frothing up. The resulting foams as well as the dry sponges had a decreased apparent density (formic, citric and tartaric acid). In contrast to this, hydrochloric and lactic acid increased the viscosity of gelatin–chitosan solutions as well as the sponge hardness.

The reaction between glutaraldehyde and chitosan or gelatin involves unprotonated amine groups (Roberts and Taylor, 1989). This reaction can be best described as a schiff basis which is slowed down in acidic media. Crosslinking times of more than 6 h do not increase the crosslinking rate any further (Tabata and Ikada, 1989). Nevertheless, lower or no crosslinking rates for chitosan in tartaric, citric and hydrochloric acid solution were observed. Chitosan–glutaraldehyde solutions have a yellow brown color. Increase in color strength represents an increased rate of crosslinking (Roberts and Taylor, 1989). Solutions containing chitosan, glutaraldehyde in citric or tartaric acid were as pale as uncrosslinked solu-

Table 3
Residual acid contents in sponges

Acid type	Acid concentration (%w/w)	Acid content in dry product (% w/w)		S.D. (% w/w)	Acid content in dry product (mmol/g)
	In bulk solution	Calculated	Measured		
Acetic acid	0.5	3.9	2.5	0.08	0.42
	1	7.5	3.2	0.15	0.53
	2	13.9	3.3	0.48	0.55
	4	24.5	3.6	0.42	0.60
Formic acid	0.5	3.9	2.9	0.11	0.62
Lactic acid	1	7.5	6.2	0.38	0.69
Hydrochloric acid	0.26	2.0	2.6	0.03	0.70
Tartaric acid	4	24.5	24.3	1.06	1.62
Citric acid	3	19.6	17.9	0.10	0.93

tions. The pH value of 1% chitosan in 0.26% hydrochloric acid was the lowest one (1.85), so for the described reasons no crosslinking was accomplished with solutions containing this acid.

In uncrosslinked sponges, the release of prednisolone was accelerated with increasing apparent density, and hydrochloric and lactic acid showed the fastest release rates. It is worth noting that not only the release rate but also the shape of the plot is changed by the acid type. This could be explained by the different degree of complexation. Crosslinked sponges showed an opposite trend: crosslinking caused an increased apparent density and a decreased rate of drug release.

However, a marked effect of the acid type on the drug liberation was noted, no correlation between residual acid content in the sponge (dry) and dissolution rate of the drug was observed. Moreover, neither a correlation between the acid strength (i.e. the pK_a value) and the drug release could be detected.

To sum up, the dissolution of prednisolone from chitosan–gelatin sponges is a function of several different factors: the rate of complexation between chitosan and gelatin, the viscosity of the polymer solution and the swollen polymer, the apparent density and, therefore, the wettable surface, the incorporated solvents (acids) and the degree of crosslinking.

5. Conclusion

Both, drug release and mechanical properties of chitosan–gelatin sponges, could be manipulated by the choice of acid type without any other additives. There was no direct correlation between the acid amount, the molar acid concentration or the acid strength and release characteristics. The acid type was of higher importance than the amount of acid for the drug liberation. Sustained release could be effected by using formic and acetic acid. Fast release and disintegration was realisable by using tartaric or citric acid. Fast release and stable sponges were achieved with hydrochloric and lactic acid.

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