TECHNOLOGY OF PREPARATION AND EVALUATION OF KETOPROFEN-CHITOSAN SOLID DISPERSION

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Abstract
The aim of the study was to investigate the effect of chitosan on the dissolution of ketoprofen incorporated into this polymer carrier. The study investigated ketoprofen in physical mixtures at the drug to polymer ratios of 1:9, 3:7, and 5:5. The solubility investigation was performed by means of a dynamic method in a dissolution apparatus; the mean amount of dissolved ketoprofen and the drug to polymer quantitative ratio in which the solid dispersion possessed the most beneficial properties improving the drug solubility were calculated. The study revealed a multi-fold increase (33 times) in ketoprofen solubility in the presence of chitosan, which increased with duration of the study and with increasing percentage of the polymer in formulations. The dissolution rates of ketoprofen in the presence of chitosan at the weight ratio 1:9 increased with the decrease of the molecular weight of the chitosan. The results obtained may help to develop new technologies for ketoprofen preparations with chitosan, with better solubility characteristics, and thus increased bioavailability of the drug.

Key words: *solid state, dissolution, ketoprofen, molecular weight of the chitosan.*

1. Introduction

Solubility and permeability are the fundamental parameters controlling the rate and extent of drug absorption. Amidon and co-workers devised a Biopharmaceutical Classification System (BCS) that categorised drugs into four classes according to their solubility and permeability properties. The objective of the BCS is to predict the *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility [1, 2].

Ketoprofen is also known as 2-(3-benzoyl phenyl) propionic acid. It has anti-inflammatory, analgesic and antipyretic effects, and is used in a wide variety of acute and chronic inflammatory diseases and in the treatment of rheumatoid arthritis.

Ketoprofen belongs to class II of the BCS, which means that its low water solubility is the limiting step for absorption and bioavailability. Several methods have been used to improve the oral bioavailability of poorly soluble drugs as an example solid dispersion technique with water soluble carriers. The increase in the dissolution rate of poorly water soluble drugs from solid dispersions can be attributed to one or a combination of different factors [3, 4].

Solid dispersion is one of these methods, and involves the dispersion of drugs in an inert carrier in the solid state prepared by melting, solvent or solvent-melting method.

Solid dispersions with the use of polymers, especially chitosan, as a carrier, play an exceptional role. Chitosan, dispersing in water environments, causes a significant increase in the contact area of the drug with solution, increases its hydrophilic properties and may affect its crystalline structure. All of these factors lead to increased solubility of the drug [5, 6].

Thus, a study was undertaken to investigate the effect of chitosan on the solubility of ketoprofen incorporated into this polymer carrier. Studies were carried out for different viscosity and average molecular weights of chitosan.

Demonstration of the effect of chitosan on the average molecular weight in various formulations or with various methods of preparation of the solid dispersions on the dissolution of ketoprofen may enable the development of new preparations of this drug with increased solubility.

2. Materials and methods

2.1. Materials

The study was performed with the use of ketoprofen (Ketoprofen p.a. min. 99%, Zhejiang Chemicals) incorporated into natural, highly purified chitosan **sample S** with 92% deacetylation and viscosity average molecular weight M_η = 1087 kDa, intrinsic viscosity η = 0.7437 [$dm^3\ g^{-1}$], **sample A** 92% deacetylation and viscosity average molecular weight M_η = 839 kDa, intrinsic viscosity η = 0.5843 [$dm^3\ g^{-1}$], **sample B** 92% deacetylation and viscosity average molecular weight M_η = 407 kDa, intrinsic viscosity η = 0.2986 [$dm^3\ g^{-1}$]

(Chitosan Huasu p.a., Chitin, France), sodium lauryl sulphate p.a., PPH "Stanlab", Poland, Aqua purification, acc. to FP IX.

2.2. Methods

2.2.1. Examination of pure ketoprofen and its physical mixtures dissolution rate

Evaluation of dissolution was performed in a dissolution apparatus according to FP IX, which describes the investigation of active substance solubility rate from solid drug forms [7]. The examination was performed in a VanKel VK 7025 dissolution apparatus, to which a Varian Inc. fraction collector was attached. Here, 1000 ml of 0.1 M hydrochloric acid at pH 1.5 was used as a release medium.

Solubility was evaluated after compressing 100 mg of samples, which were placed in each of the six chambers of the apparatus at 37 ± 0.5 °C, with velocity of 90 rotations per minute. The trial was continued for 1 hour, with 5 ml samples collected at 10 time intervals, i.e. after 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 minutes. Collected samples were filtered using filters with a pore size of 10 μm.

The collected samples were diluted and next their content was evaluated with the use of a JASCO V650 spectrophotometer with a 1 cm cuvette at a wavelength of $\lambda = 260$ nm.

The drug concentration in samples and an average percentage of dissolved ketoprofen were calculated using a linear regression equation for ketoprofen, $y = 0.06028\ x + 0.0321$. Quantitative drug-to-polymer ratios in which the solid dispersion had the most beneficial properties improving drug solubility were determined.

2.2.2. Technology for the preparation of investigated formulations

2.2.2.1. Preparation of samples for investigation of ketoprofen dissolution

The dissolution of ketoprofen was investigated immediately after compressing the powders in a Specac hydraulic press. The 100 mg samples weighed on a Mettler balance were pressed on a punch die with a diameter of 13 mm. The pure drug and physical mixtures were pressed at a pressure of 5 ton for 10 sec.

2.2.2.2. Preparation of ketoprofen and polymer physical mixtures.

Physical mixtures were prepared by grinding adequate amounts of ketoprofen in an agate mortar for 10 minutes with chitosan with an average molecular weight of: $M_\eta = 1087$ kDa, $M_\eta = 839$ kDa, $M_\eta = 407$ kDa at drug-to-polymer weight ratios of 1:9, 3:7 and 5:5 weighed on a Sartorius analytical balance. The prepared physical mixtures were passed through a sieve with 315 μm holes, and next placed in glass bottles sealed with cork and stored in an exicator over silica gel. Every sample was prepared in 1 g amount (***Table 1***).

2.2.3. Statistical analysis

Statistical analysis was performed for the maximum percentage values of ketoprofen solubility (in 60 minutes) from dispersions containing chitosan. The effects of chitosan as well as the effect of the dispersion preparation method on the drug solubility were analysed. In order to check the normality of the distribution of variables, the following tests were performed: Kolmogorov-Smirnov, Lilliefors, and the Shapiro-Wilk tests. Next, the variance homogeneity

Table 1. *The quantitative composition of physical mixtures of the ketoprofen with chitosan.*

average molecular weight of chitosan	Physical mixtures	Drug/polymer ratio	Quantity of drug [mg]	Quantity of polymer [mg]
Sample S M_η = 1087 kDa	MFS55	5:5	500	500
	MFS37	3:7	300	700
	MFS19	1:9	100	900
Sample A M_η = 839 kDa,	MFA55	5:5	500	500
	MFA37	3:7	300	700
	MFA19	1:9	100	900
Sample B M_η = 407 kDa	MFB55	5:5	500	500
	MFB37	3:7	300	700
	MFB19	1:9	100	900

was checked by means of the Brown-Forsythe's test at a significance level $p < 0.05$; ANOVA variance analysis was also performed.

3. Results and discussion

Table 2 presents the solubility of pure ketoprofen without the presence of chitosan prepared according to p. 2.2.1. The solubility findings of pure ketoprofen in 0.1 n solution HCl were used as a reference to compare the solubility of the drug incorporated into chitosan.

The drug solubility was found to increase gradually, accounting for 0.1% to 3.05% of the investigated dose.

Analysis of the data included in ***Tables 2 - 5*** and the plotted dissolution curves shown in ***Figure 1*** revealed that the addition of chitosan had a beneficial effect on the ketoprofen dissolution profile in the investigated physical mixtures.

The results of the study demonstrated that all of the investigated physical dispersions of ketoprofen with chitosan increased the solubility of this drug.

Table 2. *Dissolution of ketoprofen alone in 0.1 n solution HCl.*

Time [min]	Average % of dissolved ketoprofen	Standard deviation
5	0.10	0.024
10	0.19	0.073
15	0.28	0.054
20	0.42	0.089
25	0.56	0.092
30	0.67	0.102
35	0.89	0.127
40	1.43	0.117
50	2.09	0.104
60	3.05	0.103

Table 3. *Influence of chitosan S (average molecular weight M_η = 1087 kDa) on the dissolution of ketoprofen from physical mixture in 0.1 n aqueous solution HCl.*

Time [min]	Ketoprofen - to - Chitosan S weight ratio of physical mixtures					
	MFA 5:5		MFA 3:7		MFA 1:9	
	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved ketoprofen	Standard deviation
5	32.04	0.117	48.12	0.090	71.09	1.3223
10	47.11	0.434	75.35	0.306	87.57	1.3053
15	64.32	1.551	85.95	0.385	90.83	1.3223
20	82.53	0.289	95.08	0.585	94.51	1.0483
25	90.24	0.166	95.53	0.547	96.01	0.9992
30	94.89	0.203	95.68	0.960	96.04	0.9651
35	95.26	0.098	96.02	0.369	96.82	0.8979
40	96.57	0.001	96.44	0.231	97.27	0.9981
50	96.65	0.057	97.02	0.208	97.36	0.6996
60	97.06	0.067	97.29	0.191	98.45	0.5940

Table 4. *Influence of chitosan A (average molecular weight M_η= 839 kDa) on the dissolution of ketoprofen from physical mixture in 0.1 n aqueous solution of hydrochloric acid.*

Time [min]	Ketoprofen - to - Chitosan A weight ratio of physical mixtures					
	MFA 5:5		MFA 3:7		MFA 1:9	
	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved Ketoprofen	Standard deviation
5	37.45	0.623	56.72	3.771	75.54	0.100
10	56.54	0.060	83.57	0.568	90.53	0.728
15	69.34	0.636	89.91	0.272	92.18	1.514
20	80.51	0.793	91.91	0.935	93.39	0.644
25	85.97	0.909	93.30	0.138	94.07	0.037
30	89.93	0.787	95.11	0.150	95.12	0.807
35	92.37	0.974	95.59	0.444	95.61	1.122
40	94.02	1.269	95.86	0.301	96.29	0.775
50	96.02	0.123	96.68	0.368	97.15	0.021
60	96.17	0.096	97.67	0.296	98.55	0.027

The solubility findings of pure ketoprofen in 0.1 n HCl were used as reference to compare the solubility of the drug incorporated into chitosan. The drug dissolution was found to increase gradually and ranged from 0.1% at 5 min to 3.05% at 60 min for the investigated dose.

The presence of chitosan significantly increased the solubility of ketoprofen, which increased with duration of the trial and with increasing percentage of polymer in the formulations.

The dissolution of ketoprofen, amounting to 71%, was observed after 5 minutes from physical mixtures containing the drug-to-polymer weight ratio 1:9 in the presence of chi-

Table 5. *Influence of chitosan B (average molecular weight M_η = 407 kDa) on the dissolution of ketoprofen from physical mixture in 0.1 n aqueous solution hydrochloric acid.*

Time [min]	Ketoprofen - to - Chitosan B weight ratio of physical mixtures					
	MFB 5:5		MFB 3:7		MFB 1:9	
	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved ketoprofen	Standard deviation	Average % of dissolved ketoprofen	Standard deviation
5	42.82	0.1356	61.96	0.3324	81.95	0.5707
10	60.83	0.0674	84.86	0.1193	91.92	0.8857
15	68.83	0.1551	92.48	0.1490	92.91	0.7524
20	75.29	0.0189	93.21	0.1572	93.97	0.6089
25	81.83	0.0171	93.80	0.2174	96.53	0.6393
30	86.75	0.2131	93.99	0.2002	96.71	0.7376
35	89.77	0.4177	94.16	0.2682	97.96	0.6290
40	92.37	0.1961	95.04	0.3222	98.32	0.7695
50	96.15	0.7721	97.28	0.2956	98.62	0.8458
60	98.01	0.5706	98.29	0.2925	99.45	0.6756

tosan S. In dispersions containing 30% of the drug and polymer, the solubility of ketoprofen was observed to reach a level of 48% after 5 minutes. The lowest solubility was observed in dispersions in which the drug-to-polymer weight ratio was 5:5, in which case the drug solubility was slightly above 32%.

The highest dissolution of ketoprofen, amounting to 99%, was observed after 60 minutes from physical mixtures containing the different drug-to-polymer weight ratios in the presence of chitosan B.

Comparing data from ***Tables 3*** and ***Figure 1***, we can see a significant increase in the drug solubility, which increased almost 33 times in the presence of chitosan in comparison to the solubility of pure drug.

When the course of ketoprofen solubility curves in the presence of chitosan is observed, it becomes apparent that they are situated in the field above the ketoprofen solubility curve without polymer. The solubility curve for drug mixed with chitosan S at a ratio of 1:9 assumes the highest position in the field; also, the inclination angle of the straight line to time axis is significant, and the drug solubility in relation to time ranges from 71.09% to 98.45%, i.e. it increases with time.

The solubility line of pure ketoprofen is characterised by a low inclination angle to the time axis, and the drug solubility in time increases slightly from 0.1% to 3.05%. The increased solubility of ketoprofen in physical mixtures with chitosan may be explained by numerous factors; the decreased size of the molecules may be the first. Chitosan, when dispersed in water, may cause molecular dispersion of the drug by increasing the surface of the drug solubility [8].

Comparison of data from ***Tables 2 - 5*** and ***Figure 1*** demonstrates a significant increase in drug solubility, which in the presence of chitosan - Mη = 1087 kDa (sample MFS) in

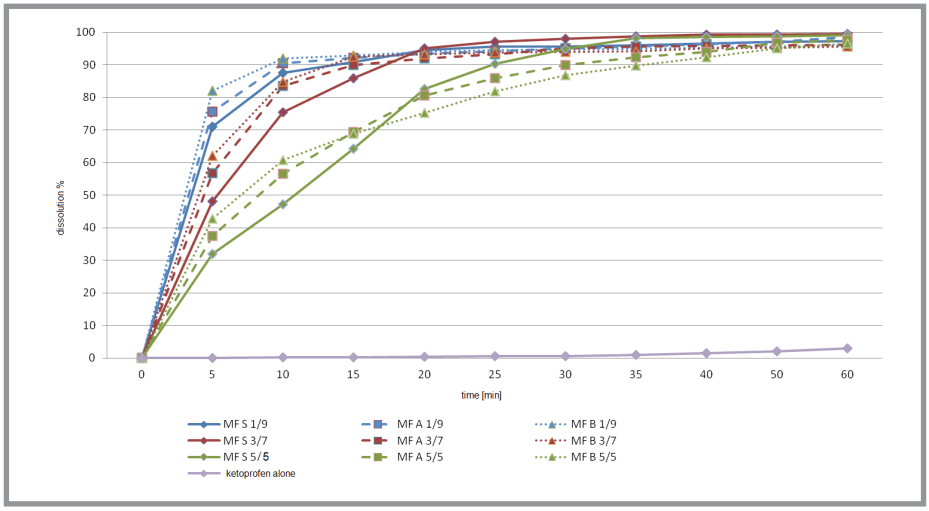

Figure 1. *Dissolution profiles of ketoprofen from physical mixture in in 0.1 M aqueous solution hydrochloric acid.*

dispersions, was almost 33 times higher in relation to the amount of added polymer in comparison to the solubility of pure drug. It was noticed that the dissolution rate of ketoprofen from the dispersions 1:9 in the presence of chitosan - Mη = 839 kDa (sample MFA) and Mη = 407 kDa (sample MFB) at 5 minutes was obviously higher than that from the dispersions with chitosan - Mη = 1087 kDa.

The enhancement of drug dissolution rate is 33-fold for the weight ratio 1:9 solid dispersion ketoprofen in chitosan - Mη = 839 kDa and chitosan - Mη = 407 kDa.

In dispersions containing 10% of the drug and 90% chitosan with an average molecular weight of approximately 839 kDa, the solubility of ketoprofen was dependent on time from 75.5 to 98.5%. The highest solubility was observed in dispersions in which the drug was incorporated in chitosan with an average molecular weight of approximately 407 kDa, in which case the drug solubility was dependent on time from 81.95 to 99.45%. The results demonstrated higher drug dissolution of the physical mixtures containing chitosan sample B than that of chitosan sample A and sample S.

Chitosan in dispersions may prevent the agglomeration of ketoprofen molecules and increase the wettability of drug molecules, thus intensifying drug solubility. The above findings were supported by statistical analysis of the investigated samples.

Normality tests performed for the percentage amount of dissolved ketoprofen after 60 minutes in relation to the drug-to-polymer weight ratio revealed the normal distribution of data. Variance analysis demonstrated statistically significant differences in the findings in relation to the applied composition of physical mixtures. Values $p<0.05$ were assumed to be statistically significant.

4. Conclusions

1. Chitosan significantly increased the dissolution rate of ketoprofen.

2. The effect depends on the drug-to-polymer quantitative ratio. The highest solubility of ketoprofen was achieved in the presence of chitosan at the drug-to-polymer weight ratio 1:9 (10% drug and 90% polymer). The dissolution enhancement in physical mixture of the drug-to-polymer weight ratio 1:9 was ~ 30 times higher than that of ketoprofen alone.

3. The effect depends on the techniques used for the preparation of physical mixtures and on the molecular weight of chitosan. The dissolution rates of ketoprofen in the presence of chitosan at the weight ratio 1:9 increased with a decrease in the molecular weight of chitosan.

4. The above results may enable the development of new technologies for ketoprofen formulations with the use of chitosan, which would be characterised by better solubility and thus the increased bioavailability of the drug.

5. References

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