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Influence of formulation properties on chemical stability of captopril in aqueous preparations

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The influence of various formulation properties on the chemical stability of captopril in aqueous media at pH 3 was investigated, in order to reformulate and increase the shelf-life of an oral mixture of the drug. At this pH, chemical stability is improved by an increase in drug concentration (1–5 mg/ml) and a decrease in temperature (5–36 °C), the latter demonstrated by a linear Arrhenius-plot. The activation energy is low ($E_a = 10.2$ kcal/mol), thus the Q_{10} value is only 1.8 in pure aqueous solutions. The degradation at the lowest concentration investigated in pure aqueous solution apparently follows zero order kinetics. The reaction order is changed at higher concentrations. We are presenting a hypothesis of intramolecular proton transfer from the thiol to the ionized carboxylic group as the initial step in the oxidative degradation pathways of captopril.

Long-term stability of 1 mg/ml captopril in aqueous solutions at pH 3, stored at 36 °C for one year, shows that the sugar alcohol sorbitol accelerates degradation of the drug while Na-EDTA at a concentration as low as 0.01% is sufficient to stabilize these samples. Purging with N_2-gas prior to storage is not essential for drug stability, as long as Na-EDTA is present. Only at a low level of Na-EDTA (0.01%) combined with a high level of sorbitol (35%), purging with N_2-gas appears to have a small effect. The destabilizing effect of sugar alcohols is confirmed by accelerated degradation also in the presence of glycerol. The efficient stabilization in the presence of Na-EDTA at a low concentration indicates that the metal-ion-catalyzed oxidation pathway dominates the chemical degradation process at low pH, although several mechanisms seem to be involved depending on excipients present.

1. Introduction

Captopril was brought into the market in the early 1980ies (Kadin 1982), and has become one of the ACE-inhibitors most frequently used for children suffering from hypertension and heart failure. It is the preferred drug for infants during their first months of living (Grenier et al. 2000). The dose can be as low as 10 μg/kg in certain cases, e.g. for premature, newborn, or at lowered kidney-function (Martin 2007). The hospital pharmacies produce oral aqueous mixtures of the drug, in order to provide a flexible dosage form that can be administered to infants.

Captopril is a thiol (Scheme) that undergoes oxidative degradation to captopril disulfide in aqueous solutions. The reaction is free-radical-initiated, involving complex mechanisms of autooxidation and metal-ion-catalyzed oxidation. Degradation in solution is accelerated above pH 4. In the solid state, pure captopril exhibits excellent stability, but is readily oxidized in the presence of excipients that release moisture (Kadin 1982; Timmins et al. 1982; Connors et al. 1986). At present, the oral mixtures that are produced in our hospital pharmacy has a shelf-life of four months refrigerated and should be used within four weeks after breakage.

The aim of this study was to investigate the influence of various formulation properties on the chemical stability of captopril in acidic aqueous solutions. The results will be used to reformulate captopril oral solutions, in order to increase shelf-life. Reformulation into a stabilized preparation will increase quality and safety of the product, and is essential for a centralized production of captopril mixture. As maximum stability of captopril is obtained in acidic solutions, we selected pH 3 for our prospective preparations and experimental setup. Concentrations of the mixtures that are produced in our hospital pharmacy at present (1 and 5 mg/ml) determined the selected concentration range of the study.

2. Investigations, results and discussion

2.1. Concentration dependent stability in buffer-free media at pH 3

There is a need for evaluation of concentration dependent degradation at low pH in buffer-free media, as previous studies were performed under basic and neutral conditions (Kadin 1982; Lee and Notari 1987) or at low pH in the presence of phosphate buffer (Mathew and Das Gupta

1996). Chemical stability of captopril in water for injections at pH 3/25 °C is improved by an increase in drug concentration, as expected (Table 1). The degradation at the lowest concentration investigated (1 mg/ml) seems to follow zero order degradation ($R^2 = 0.967$), while the degradation kinetics is changed at higher concentrations. This is consistent with results obtained at neutral pH (Lee and Notari 1987). The degradation of captopril 2.5 mg/ml seems to run by a fast initial phase followed by a slower phase, but apparently does not fit well a first order model ($R^2 = 0.893$). Degradation at 5 mg/ml was not followed long enough to thoroughly predict the apparent reaction order (Sande 1996). A change in reaction kinetics by a change in drug concentration is not unexpected, considering the complexity of the reaction mechanisms involved (Connors et al. 1986). The shelf-life of captopril 1 mg/ml in water at pH 3, expressed as t_{90} (10% decomposition) is only 7 days, assuming zero order degradation. A low pH is not sufficient to stabilize the drug in aqueous preparations. An increase in drug concentration to 2.5 mg/ml raises the shelf-life four to five times, while the shelf-life is increased seven to eight times by increasing the concentration to 5 mg/ml (Table 1).

Precipitates as "black spots" were observed in the 5 mg/ml samples after 57 days storage at 25 °C, while the phenomenon occurred after 73 days in the 1 and 2.5 mg/ml samples. Chan et al. (1994) made the same observations, which can be precipitates of the main degradation product captopril disulfide with a lower aqueous solubility than the drug. The captopril 1 mg/ml oral solution is needed to provide flexible dosages for the smallest infants. It also has the shortest shelf-life (Table 1). The 1 mg/ml solution was thus selected for further evaluation.

2.2. Temperature dependent stability at pH 3

The shelf-life (t_{90}) of captopril 1 mg/ml in aqueous solution at pH 3 (no excipients added) is increased from 7 days at room temperature (25 °C) to 26 days by storage in the refrigerator (5 °C), and reduced to 4 days by storage at elevated temperature (36 °C), calculated by zero order degradation kinetics (Table 1). The results are in accordance with data from Pereira and Tam (1992). The pH remained constant and the loss of solvent was insignificant throughout the study (63 days). The experimental data give a linear plot ($R^2 = 0.999$) by use of the Arrhenius eq. ($A = 1.8 \cdot 10^4$ mg/ml $\cdot h^{-1}$, $E_a = 42.8$ kJ/mol). Changes in temperature have a limited influence on the degradation rate of captopril at pH 3, compared to a group of representative drug substances (Yoshioka and Stella 2000). As the activation energy is low, the Q_{10} value (the factor by which the rate constant of the oxidative degradation changes for a 10 °C change in temperature) is only 1.8 ± 0.1, estimated by using the calculated self-lives in Table 1 according to the equation given by Longland and Rowbotham (1989).

The content of molecular oxygen in water is significantly decreased with a rise in temperature under atmospheric conditions (Connors et al. 1986), but the degradation seems to be independent of changes in the oxygen content within the experimental range. This is reflected in the measured consumption of oxygen during storage, which proceeds independently of the degradation rate (see Section 2.7.). The further experiments were performed at 36 °C, in order to reduce experimental time. The apparent degradation rates were calculated according to zero order reaction kinetics.

2.3. Postulate of intramolecular proton transfer as initial degradation step

Captopril contains two ionisable functional groups. Although the thiol group (pKa = 9.8) is fully protonated at low pH, the postulated reaction mechanisms for autooxidation and metal-ion catalyzed oxidation are based on initial ionization of the thiol group only. Water is the suggested proton acceptor in the reaction, even though it is a weak nucleophile (Timmins et al. 1982). Formation of the reactive thiolate by an intramolecular proton transfer to the deprotonated carboxylic group (pKa = 3.7) can explain the pH-rate profile for oxidation of captopril, where optimal stability is obtained below pH 3.5 (Timmins et al. 1982; Kadin 1982). Intramolecular hydrogen bonding between the functional groups is likely, and will favour the reaction as illustrated in the Scheme. At pH 3.0 (experimental conditions) about 17% of the carboxylic groups are deprotonated, i.e. sufficient to act as a driving force in the degradation reaction. Intramolecular hydrogen bonding has been suggested to take place between the carboxylic acid hydrogen and the amide carbonyl oxygen, but only in the trans-form of the captopril molecule (Rabenstein and Isab 1982).

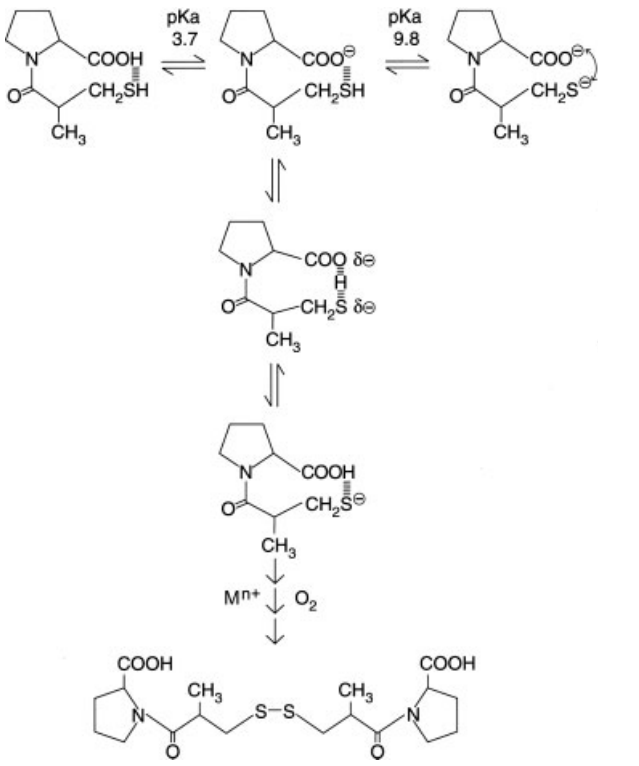
Inclusion of the thiol group in the cavity of α-cyclodextrin protects captopril from oxidative degradation, while inclusion of the whole captopril molecule in the cavity of β-cyclodextrins does not change the degradation rate to any extent (Ikeda et al. 2002). Complexation with α-cyclodextrin will separate the thiol from the carboxylic group, while shielding of these polar groups by intramolecular bonding and subsequent proton transfer will be favoured in the lipophilic cavity of β-cyclodextrins. The results support the hypothesis of proton transfer from thiol to the carboxylic group as the initial step in the oxidative degradation pathways of captopril.

Table 1: Thermal degradation of captopril in water for injection at pH 3, as a function of drug concentration and storage temperature

Captopril concentration (mg/ml)	Temperature (°C)	k_0 (mg/ml $\cdot h^{-1}$)	R^2	t_{90} (days)	$t_{1/2}$ (days)	k_1 (h^{-1})	R^2	t_{90} (days)	$t_{1/2}$ (days)
	5	$1.6 \cdot 10^{-4}$	0.960	26	128				
1.0	**25**	$5.7 \cdot 10^{-4}$	0.967	7	36	$9.7 \cdot 10^{-4}$	0.907	5	30
	36	$1.0 \cdot 10^{-3}$	0.943	4	20				
2.5	**25**	$3.1 \cdot 10^{-4}$	0.847	33	167	$1.7 \cdot 10^{-4}$	0.893	26	169
5.0	**25**	$3.9 \cdot 10^{-4}$	0.852	54	269	$9.5 \cdot 10^{-5}$	0.855	46	305

The half-life ($t_{1/2}$) and shelf-life (t_{90}; expressed at 10% decomposition) are calculated from the apparent zero order (k_0) or first order (k_1) degradation rate constants

Scheme



Postulated initial mechanisms for the oxidation of captopril into caotopril disulfide, via formation of the reactive thiolate by an intramolecular proton transfer to the deprotonated carboxylic group.

2.4. Influence of solvent properties on drug stability

Chemical stability of captopril seems to be dependent on the amphiprotic properties of polar solvents (water, ethanol, isopropanol). There is a linear increase ($R^2 = 1.000$) in the apparent zero order degradation rate constant (k_0) with an increase in solvent proton donor properties (measured as the α parameter), as well as a linear decrease ($R^2 = 0.996$) in k_0 with an increase in solvent proton acceptor properties (as measured by the β parameter; Kamlet et al. 1983; Table 2). Amphiprotic solvents can interact with functional groups of the solute by both proton donor- and acceptor mechanisms during continually forming and reforming interactions. The importance of the relative acidic/basic properties of the solvent was evaluated by a plot of k_0 versus α/β, showing that the degradation rate of captopril is increased linearly with an increase in relative acidity of the solvent ($R^2 = 0.986$). As correlating observations, oxidation of captopril is reported to occur less readily in methanol ($\alpha = 0.93$; $\beta = 0.62$; $\pi^* = 0.60$) than in aqueous solution (Kadin 1982), and addition of cosolvents is reported to improve the stability of captopril in aqueous systems (Mathew and Das Gupta 1996).

The results are contradictory to the postulated ionization of the thiol function into a reactive thiolate by water as a proton-acceptor (Timmins et al. 1982), and strengthen our hypothesis of the carboxylate group as an intramolecular proton acceptor in the oxidative degradation pathways. The influence of proton donor properties on drug stability can (partly) depend on solvent reactions with the peroxyldianion (O_2^{2-}) that is produced during autooxidation as well as the metal-ion catalyzed pathway (Timmins et al. 1982). Protonation will promote the formation of hydrogen peroxide ($O_2^{2-} + 2H^+ \rightarrow H_2O_2$) and hydroxyl radicals ($H_2O_2 \rightarrow 2OH^{\cdot}$). These highly reactive oxidative species will propagate the chain reactions ($RS^- + OH^{\cdot} \rightarrow RS^{\cdot} + OH^-$) and lead to further degradation of the drug by oxidative reactions (Halliwell and Gutteridge 1999).

Polarity of the solvents can also be important, as radical reactions are favoured in strongly polar solvents such as water. Increased solvent polarity, measured as the π^* parameter (Kamlet et al. 1983), leads to an increase in k_0 (Table 2). However, the lower apparent degradation rate in isopropanol compared to ethanol cannot be explained by the small differences in polarity only. Secondary alcohols, as isopropanol, are good scavengers of free radicals, and will often protect the therapeutic substance from degradation (Moore 1996). This can be a property that is important for the stability of

Table 2: Thermal degradation of captopril (1 mg/ml) in pure protic solvents or in aqueous solutions containing various excipients, studied at 36 °C

Vehicle	k_0 (mg/ml · h^{-1})	R^2	$t_{1/2}$ (days)	Properties of vehicle		
				α	β	π^*
Water	$1.0 \cdot 10^{-3}$	0.943	21	1.17	0.18	1.09
Ethanol	$4.5 \cdot 10^{-4}$	0.870	47	0.83	0.77	0.54
Isopropanol	$3.2 \cdot 10^{-4}$	0.922	65	0.76	0.95	0.48
					pH	
Glycerol 20%	$1.3 \cdot 10^{-3}$	0.717	17		3.0	
Glycerol 50%	$1.6 \cdot 10^{-3}$	0.771	13		3.0	
Glycerol 80%	$6.0 \cdot 10^{-4}$	0.697	33		3.0–3.4	
					pH	
NaCl 0.001 M	$1.3 \cdot 10^{-3}$	0.934	16		3.0	
NaCl 0.010 M	$1.3 \cdot 10^{-3}$	0.893	17		3.0	
NaCl 0.100 M	$2.5 \cdot 10^{-3}$	0.914	8		3.0	
					pH	
Citrate buffer 0.010 M	$2.1 \cdot 10^{-3}$	0.847	10		3.0	
Phosphate buffer 0.010 M	$1.7 \cdot 10^{-3}$	0.968	12		3.0	
					pH	
Na-EDTA 0.010%	ND (10 d.)				3.0	
Na-EDTA 0.100%	ND (10 d.)				3.3	

The half-life ($t_{1/2}$) is calculated from the apparent zero order degradation rate constant (k_0). Abbreviations: α = solvent proton donor properties, β = solvent proton acceptor properties, π^* = solvent polarity, ND = no degradation detected after 10 days of storage

Table 3: Long-term stability of captopril (1 mg/ml) in aqueous solution at pH 3 containing various excipients, expressed as % drug remaining after storage at 36 °C

Sample	Excipient				Days of storage								
	N_2-gas	Sorbitol %	Na-EDTA%	pH	0	3	8	15	29	49	97	188	365
					Captopril (%) remaining after storage								
A	+	20	0.010	3.0	100	98	101	103	99	100	102	96	93
B	+	20	0.100	3.3	100	98	99	100	98	101	100	91	96
C	+	35	0.010	3.0	100	100	101	97	95	100	98	101	97
D	+	35	0.100	3.3	100	99	99	98	96	98	100	97	96
E	−	20	0.010	3.0	100	98	97	98	98	100	102	96	94
F	−	20	0.100	3.3	100	97	95	97	96	98	100	97	95
G	−	35	0.010	3.0	100	96	98	96	95	98	98	94	89
H	−	35	0.100	3.3	100	96	96	96	96	107	98	95	92
I	−	0	0	3.0	100	89	77	66	43	22	15	0	0
J	−	20	0	3.0	100	89	73	49	28	13	5	0	0

captopril, as changing the solvent from water ($\pi^* = 1.09$) to ethanol ($\pi^* = 0.54$) doubles $t_{1/2}$, while changing the solvent to isopropanol ($\pi^* = 0.48$) triples $t_{1/2}$ (Table 2).

The degradation rate of captopril is possibly influenced by the viscosity of the vehicle, due to increased lifetimes of free radicals, reduced diffusion distances and changed reactivity of short lived intermediates in viscous media. Our results indicate that other solvent properties are more important than viscosity, as increasing the relative viscosity by a factor 1.3 when changing the medium from water to ethanol has a much larger effect on k_0 (reduced by 57%) than a further increase in relative viscosity (factor 1.4) when changing the medium from ethanol to isopropanol, which reduces k_0 by 28% (Table 2). Further evaluation of the influence of viscosity by use of a glycerol gradient (0–80%) resulted in a linear increase in k_0 as a function of increased measured viscosity in the concentration range 0–50% glycerol ($R^2 = 0.973$), but a significant drop in k_0 at a high glycerol concentration (80%). Other mechanisms than viscosity thus seem to influence at glycerol concentrations $\leq$50%, until viscosity can become important and retard degradation at a certain level. Glycerol is a sugar-alcohol that can act as a proton donor e.g. in reactions with O_2^{2-}. The sugar alcohol sorbitol has a similar effect on drug degradation (Section 2.10; Table 3, Sample J versus I).

Water is of course the vehicle of choice, and addition of co-solvents is restricted in paediatric preparations. According to our results, the amphiprotic properties (relative acidity) and the radical scavenging properties of excipients in the formulation is more important than the influence of polarity. Viscosity seems to be a property of minor concern.

2.5. Primary kinetic salt effect at pH 3

Chemical stability of captopril at pH 3 is dependent on the ionic strength (μ) of the solution (Table 2), evaluated in water ($\mu \sim 0$) and aqueous buffer-free saline solutions at $\mu = 0.001$–0.1. No change in pH was detected during the experimental time (10 days). We observed a positive primary kinetic salt effect in the degradation of captopril at acidic pH. A plot of log k_0 as a function of $\sqrt{\mu}$ according to the Brønsted-Bjerrum eq. is close to linearity ($R^2 = 0.955$; slope = 1.14) and $Z_A Z_B = 1.1$ assuming $2Q = 1.04$ at 36 °C (Carstensen 1970), as is a plot of log k_0 as a function of ($\sqrt{\mu}/1 + \sqrt{\mu}$) according to the modified eq. for higher values of μ ($R^2 = 0.938$; slope = 1.50) resulting in $Z_A Z_B = 1.4$. The positive slopes and $Z_A Z_B$ values close to unity imply that the reactive species at the rate determining step is the monoanion of captopril. Our results are in accordance with the positive salt effect observed in the oxidative reactions of captopril by use of oxidizing agents (Zahdeh et al. 2007), but cannot be directly compared to contradictory results of thermal stability that were detected at much higher levels of μ in aqueous buffers (Mathew and Das Gupta 1996).

The presence of salts in aqueous formulations will lead to accelerated degradation of captopril by the positive primary salt effect, possibly by solvation of the species participating in the free radical chain reactions. The half-life ($t_{1/2}$) of captopril at 36 °C is reduced up to 24% when changing the medium from pure water ($\mu \sim 0$) to low concentrations of saline (1–10 mM), and reduced 62% at 100 mM saline when compared to degradation in pure water (Table 2).

2.6. Buffer catalysis at pH 3

Citrate buffer and phosphate buffer (both 10 mM) catalyse the degradation of captopril in aqueous media at pH 3 ($\mu \approx 0.009$), which is consistent with previous observations (Mathew and Das Gupta 1996). The half-life ($t_{1/2}$) of captopril at 36 °C is decreased from 15 days in buffer-free saline $\mu = 0.009$ (calculated according to the Brønsted-Bjerrum eq.; Carstensen, 1970) to 10 days in citrate buffer and 12 days in phosphate buffer (Table 2). The catalysing effect of phosphate buffer is previously observed also at pH 6, and is ascribed to an increased formation of the reactive thiolate by proton transfer to the phosphate anions (Chen et al. 1995). The buffer ions may also act as proton donors in reactions with O_2^{2-}, and promote degradation by the formation of $OH^{\cdot}$. In phosphate buffer, dihydrogen phosphate is the main buffer ion present at pH 3 as well as pH 6 ($pKa_1 = 2.15$; $pKa_2 = 7.09$).

Contradictory to our results, chemical stabilization by citrate buffer is reported by Chen et al. (1995) at pH 6. This can be ascribed to different amounts of citric acid and citrate anions present at acidic and neutral pH respectively ($pKa_1 = 3.13$; $pKa_2 = 4.76$; $pKa_3 = 6.40$).

2.7. Oxygen consumption at pH 3

The increased degradation of captopril when dissolved in citrate buffer at pH 3 compared to the saline reference (see Section 2.6) is not reflected in the oxygen consumption measured under the same conditions. The oxygen consumption is nearly parallel in the two media, and thus

appears to be independent on the rate of drug degradation. A selected level of 20% oxygen consumption was reached after 21 ± 0.5 h storage in citrate buffer versus 19 ± 0.5 h in the saline reference, calculated by plots of oxygen content (mg/l) as a function of time ($R^2 = 0.978$, both samples). These results confirm that captopril is decomposed by several pathways, depending on excipents in the formulation.

2.8. Complexation between captopril and excipients

Citrates can have a dual role as chelating agents, and influence drug stability by complex formation with metal ions or drug molecules in the formulation. An interaction between captopril and citrate/citric acid is demonstrated at pH 3 by UV-absorption measurements of the drug (92 μM) in a citrate buffer gradient (Fig.). There is a red shift (+8 nm) in the absorption maximum and the molar absorptivity is reduced to about one half when adding 10 mM citrate ($\lambda_{max} = 210$ nm, Abs = 0.415) compared to the reference in water ($\lambda_{max} = 202$ nm, Abs = 0.917). The interaction seems to be weak, as complexation did not influence the HPLC analysis, but may be sufficient to influence the observed stability of the drug (Section 2.6). A gradient of phosphate buffer at pH 3 does not influence the absorption properties of captopril.

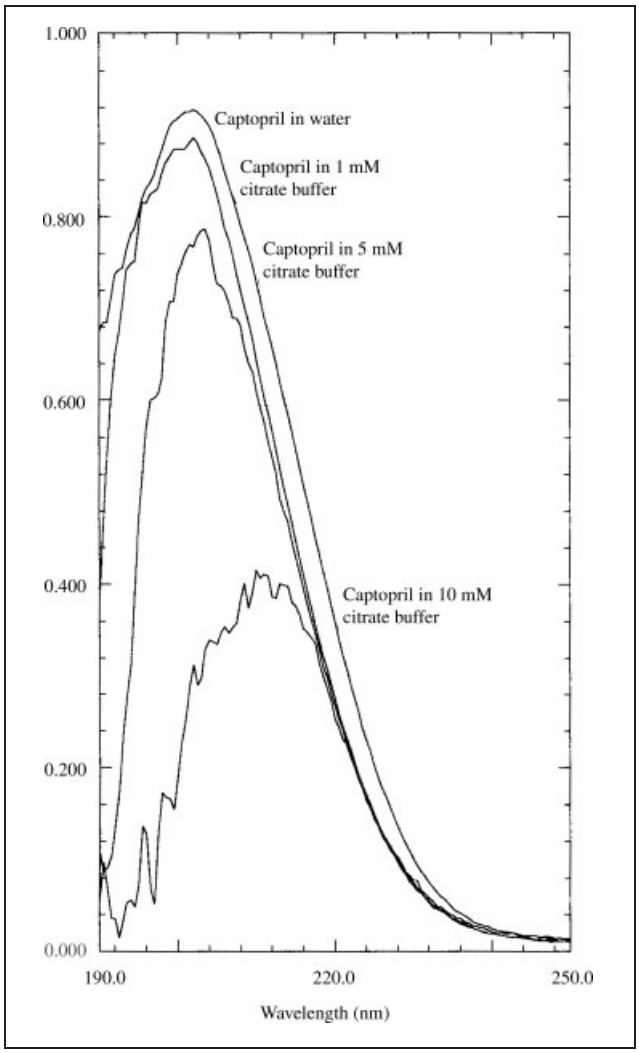


Fig.: Decrease in UV-absorption of captopril (92 μM) when changing the medium from water at pH 3 to an increasing concentration of citrate buffer at pH 3 (1; 5; 10 mM)

Investigation of ascorbic acid as an antioxidant was prohibited because of a strong interaction with captopril which influenced the HPLC analysis. Complexation was confirmed by UV-absorption measurements of captopril in an ascorbate buffer gradient at pH 3, showing the increase of a new peak at 240 nm as a function of increased buffer concentration (data not shown). As ascorbate/ascorbic acid is easily decomposed in aqueous media during storage (evaluated by UV-sbsorption measurements showing a 62% decrease in the absorption maximum at 256 nm after storage for 24 h at 36 °C), care should be taken in using ascorbic acid/ascorbate as excipients in small scale production without sufficient quantitative data concerning the influence on drug stability.

2.9. Influence of chelating agent at low concentration on drug stability

Thermal stability of captopril is known to be sensitive to the concentration of cupric ions at neutral pH (Lee and Notari 1987), and as little as 1 ppm copper is reported to catalyze captopril oxidation in solution (Kadin 1982). A long-term study performed by Berger-Gryllaki et al. (2005) show the stabilizing effect of Na-EDTA 0.1% on captopril 1 mg/ml dissolved in purified water. According to our results a much lower concentration of Na-EDTA (0.01% w/v) seems to be sufficient to stabilize captopril efficiently, as no degradation could be detected after storage of the samples at 36 °C for 10 days (Table 2). As reference, the half-life in water is 21 days at the same temperature and pH. EDTA is ionized at pH 3 ($pK_1 = 2.00$; $pK_2 = 2.67$; $pK_3 = 6.16$) and will perform metal-binding properties under acidic conditions. The efficiency of Na-EDTA implies that the metal-ion-catalyzed degradation pathway dominates the chemical degradation process of captopril at acidic pH. The metal-ion purity of the captopril drug substance is $\leq$20 ppm, thus the substance itself can be a source of metal-ions at an amount sufficient to promote degradation. Heavy metal contamination brought into the formulation by excipients is also a problem, especially for sugars, phosphate salts and citrate salts (Nema et al. 2002).

2.10. Influence of additives in combination on drug stability

Long-term stability of captopril 1 mg/ml stored at 36 °C in aqueous solution at pH 3 was evaluated for one year in the presence of Na-EDTA (0.01–0.1%), sorbitol (20–35%) or flushed with N_2-gas (Table 3). Sorbitol accelerates degradation of captopril, as demonstrated by addition of 20% sorbitol as the only excipient (Sample J versus I). The effect is similar to the effect of glycerol, as discussed in Section 2.4. Na-EDTA at concentration as low as 0.01% seems to be sufficient to stabilize the samples at long-term basis, also in the presence of 35% sorbitol (Sample A versus C; E versus G). Flushing with N_2-gas prior to storage does not seem to be essential for drug stability, as long as Na-EDTA is present (Sample A versus E; B versus F; D versus H). Only at a low level of Na-EDTA (0.01%) combined with a high level of sorbitol (35%), flushing with N_2-gas seems to have a small effect (Sample C versus G).

Na-EDTA should be administered with care to patients with heart- or kidney failure (Rowe et al. 2003), thus the concentration of the chelating agent ought to be kept as low as possible in the formulation. On the other hand, Na-

EDTA may increase metal-ion catalyzed thiol oxidation at low levels (Kadin 1982). The influence of combinations of Na-EDTA, preservatives, sweeteners and taste modifying substances on long-term stability of captopril is under further investigation in our laboratories, in order to develop a stable oral formulation.

3. Experimental

3.1. Materials

Captopril (≥98.6% pure) was purchased from Norsk medisinaldepot AS, Norway. Water for injection (Ph.Eur. quality) was used for the preparations. Milli-Q water was used for the HPLC-analysis. All other chemicals were of p.a. grade.

3.2. HPLC

Captopril was quantified by reverse phase HPLC at ambient temperature, by use of equipment from Shimadzu: SIL-10A autoinjector, GT-104 degasser, LC-10AS pump, SPD-M10A Diode Array detector, CBM-10A communication unit connected to LC-10 software, version 1.60. The column was Waters X-terra RP18, 4.6x100 mm, particle size 3.5 μm; the mobile phase consisted of methanol : water : ortho phosphoric acid 85% (38 : 62 : 0.05). UV-absorption at 220 nm was used for detection. The injection volume was 20 μl, and the flow was adjusted to 0.6 ml/min. The analytical run time for each parallel was 16 min., in order to separate captopril (retention time 3.8 min.) from the main degradation product (retention time 13.6 min.). Under these conditions, the method was linear ($R^2 > 0.998$) in the concentration range 10–70 μg/ml (n = 7), the relative standard deviation was 3.4% at 15 μg/ml (n = 6) and 1.7% at 50 μg/ml (n = 6).

3.3. UV-visible absorption spectroscopy

UV-visible absorption spectra (190–700 nm) were recorded by a Shimadzu UV-2101PC spectrophotometer.

3.4. Viscosity

Viscosity (Pa*s) was measured on a Bohlin VOR Rheometer, Sweden, equipped with a Double Gap 24/27 (volume 10 ml). The samples were measured at 36 °C by use of torque element 1.555 gcm and at share rates 36.7 s^{-1} (pure solvents) or 3.67 s^{-1} (water-glycerol mixtures). The measurements were used as relative values within each series.

3.5. Oxygen consumption

Oxygen consumption was measured at ambient temperature in an air-tight container (60 ml) without a head-space of air under continuous stirring by an Oxi 340 Oximeter connected to a CellOx 325 Dissolved Oxygen Probe equipped with a WP 90/3 membrane in an OxiCal-SL Air Calibration Beaker, adjusted for salinity by use of a LF 320 Conductivity Meter (all WTW). The oxygen level was measured at 30 min intervals for 45–90 h.

3.6. Cabinets for storage

Preparations were stored at 5 ± 3 °C in a Bosch Cooler; at 25 ± 2 °C/60 ± 5% relative humidity in a Thermax climate chamber; and at 36 ± 1 °C in a Water Jacketed incubator, Forma Scientific. The temperature and humidity were monitored with probes from Malvern, Malvern Instruments Nordic.

3.7. Preparation of samples

Captopril (n = 3) at concentration 1.0; 2.5 or 5.0 mg/ml was dissolved in water for injection; in aqueous solutions containing NaCl (1–100 mM), citrate buffer at pH 3.0 (10 mM, adjusted with NaCl to μ ≈ 0.009), phosphate buffer at pH 3.0 (10 mM; μ ≈ 0.009), Na-EDTA (0.010–0.100% w/v), glycerol (20–80% w/w); or the drug was dissolved in pure ethanol or isopropanol. The pH of the aqueous solutions was adjusted to 3.0 by addition of NaOH when appropriate, prior to storage in air-tight containers at 5, 25 or 36 °C for 10–228 days. Evaporation was controlled prior to quantification by HPLC. The pH was controlled at the last analysis of each series. The half-life ($t_{1/2}$) and shelf-life (t_{90}; expressed at 10% decomposition) were calculated by use of the estimated degradation rate constants, obtained by zero- or first order degradation plots.

Long-term stability of aqueous samples of captopril (1.0 mg/ml) at pH 3.0–3.3 (n = 8), containing sorbitol (20–35% w/v) and Na-EDTA (0.010–0.100% w/v) in combination, was studied by storage at 36 °C for 365 days. Half of the samples were flushed with N_2-gas prior to storage in air-tight containers without head space. The other samples were stored in air-tight containers with a head space of air. Samples without additives or only containing 20% sorbitol as an excipient were used as reference. The captopril concentration was quantified by HPLC at 8 defined time intervals during storage. The pH was controlled at the last analysis of each series.

Captopril (0.02 mg/ml) was dissolved in water; citrate buffer at pH 3.0 (1–10 mM); phosphate buffer at pH 3.0 (10–100 mM); or ascorbate buffer at pH 3.1 (1–10 mM) in order to evaluate complexation between the drug and buffer by UV-vis absorption measurements. The pH was adjusted by addition of NaOH when appropriate.

Captopril (1 mg/ml) was dissolved in NaCl (9 mM, pH 3.0) or citrate buffer pH 3.0 (10 mM, μ ≈ 0.009) prior to detection of oxygen consumption (n = 3).

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