We used ten independend standard mixtures to prepare the calibration matrix. The concentration range must be in the estimated range (0–120% for a dissolution procedure).
The recovery of the weighed concentrations of both substances is shown in Fig. 2. There is a good linearity in the concentration range of 0–120 mg/900 ml.
The results of Commercial product batches (tablets Evitocor® plus) are statistically equivalent to the results obtained by a HPLC method, but a better reproducibility was seen with the UV method.
Controls can be run to provide a quantitative representation of the validity of the calibration before running samples.
The method is secure, easy, fast, accurate and reproducible and much faster as a HPLC method. The validation parameters specificity, linearity, range, precision, accuracy and robustness according to ICH Q2B [2] show a good suitability of the method for the determination of dissolution of atenolol and chlortalidone in a pharmaceutical dosage form (tablets).

Experimental

1. Apparatus

A computerized UV/VIS-spectralphotometer (DU 640i, Beckman, USA) and a dissolution bath DT 70 (Erweka, FRG) coupled with a pump Minipuls (Gilson, France) are used. The measurement is done with a dissolution software (Beckman) and the multicomponent analysis with the FSQ-software (Beckman).

2. Drugs and reagents

Evitocor plus® is a product of Apogepha Arzneimittel GmbH Dresden. The tablets contain atenolol and chlortalidone as active ingredients.

3. Analytical procedure

The dissolution is determined for batch release in 5 min intervals, in the sum 30 min by UV-spectroscopy. Solvent: water 900 ml, temperature: 37°C, speed: 100 rpm, type: paddle.
The sample was pumped about filters in flow-through cells and measured with the described method using FSQ.

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Dr. Wolfgang Wehner
APOGEPHA Arzneimittel GMBH
Kyffhäuserstraße 27
D-01309 Dresden

Research Institute for Industrial Pharmacy and Departments of Pharmaceutical Chemistry and Pharmacy Practice, School of Pharmacy, Potchefstroom University for CHE, Potchefstroom, South Africa

Solubilisation of poorly water soluble non-steroidal anti-inflammatory drugs at low pH with *N*-methylglucamine

M. M. DE VILLIERS, W. LIEBENBERG, S. F. MALAN and J. J. GERBER

The objective of this study was to evaluate the solubilisation potential of *N*-methylglucamine for various non-steroidal anti-inflammatory drugs (NSAID) at low pH. The solubilising effect of *N*-methylglucamine on NSAID's represented by the arylacetic acid derivatives indomethacin and sulindac, and the arylpropionic acid derivatives naproxen, ibuprofen and ketoprofen was investigated at a pH range below the pKa values of the drugs to eliminate the effect of pH on the solubility of the drugs. The pKa values of the drugs ranged from 4.5 for indomethacin to 4.7 for sulindac. Consequently the solubility was measured at pH 3.4 and 4.2 because in this pH range the major part of the drugs is in the non-ionised insoluble form.
Previous thermodynamic and kinetic investigations of the behaviour of aqueous solutions of NSAID's reported the dominant hydrophobicity of these compounds, showing weak acidity, low solubility and dissolution rates in water, and high o/w partition coefficients, in their acidic form [1, 2]. Several approaches for solubilisation are available including techniques such as surfactant and co-solvent addition, salt formation, complexation, solid state manipulation, and prodrug derivatisation [3]. Salt formation is one of the first approaches considered as a means of increasing drug solubility and dissolution rate [4]. The most common salts of drugs, prepared to increase solubility, are the sodium and hydrochloride salts.
For acidic compounds such as NSAID's physiologically and pharmacologically compatible salts or complexes are prepared by neutralising the acidic drugs with compatible cations from corresponding inorganic and organic bases and amino acids. Pharmaceutically acceptable salts of acidic drugs are the organic, water-soluble amines made from *N*-methylglucamine (commonly known as meglumine) [5].
N-methylglucamine was first synthesised in 1935 [6]. The role of this compound in the improvement of the solubility of poorly water-soluble weak acid drugs is well known [7]. Its use in pharmaceuticals began with the preparation of meglumine antimonate for the treatment of Leishmaniasis. The effect of this weak amine salt on the solubility of drugs like salicylic acid, couvermycin A, ibuprofen and other NSAID's has been studied [5, 7, 8]. In all these studies improvement in the aqueous solubility was attributed to salt formation.
The graphical illustration of the maximum concentration of the drug as a function of *N*-methylglucamine concentration at pH 3.4 (Fig. 1) and pH 4.2 (Fig. 2) reveals the existence of nearly linear relationships (mean $R^2 = 0.977 \pm 0.023$) at concentrations of *N*-methylglucamine below 0.150 M. The concentration up to where the increase in solubility was linear was estimated from plots of an adjusted correlation coefficient against concentration as described by De Villiers et al. [9]. This phenomenon is associated with 1 : 1 complex formation [10].
At concentrations above 0.15 M and at pH 3.4 a non-linear increase in solubility of the arylpropionic acid derivatives was observed (Fig. 1). If the solubility increased lin-

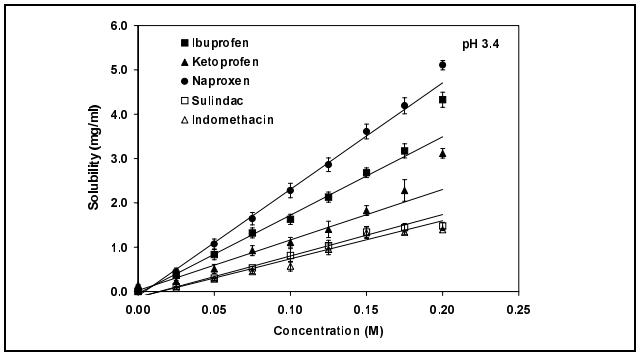

Fig. 1: Solubility of the NSAID's as a function of *N*-methylglucamine concentration at pH 3.4 and 30 °C (n = 3).

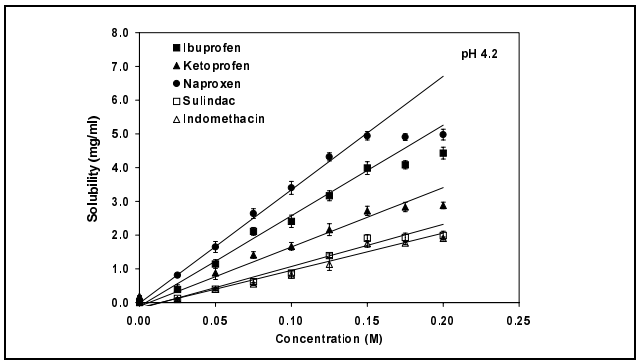

Fig. 2: Solubility of the NSAID's as a function of *N*-methylglucamine concentration at pH 4.2 and 30 °C (n = 3).

early across the concentration range studied then increases in solubility would have corresponded to the trendlines in Fig. 1. This did not happen. For example the predicted solubility for ibuprofen combined with 0.20 M *N*-methylglucamine should have been 3.49 mg/ml (y = 17.661x – 0.0441). This is significantly lower than the measured solubility of 4.33 ± 0.170 mg/ml. This indicated a greater solubilisation power at higher concentrations of *N*-methylglucamine, characteristic of hydrotrope solubilisation [11]. Hydrotrope solubilisation was probably due to the formation of higher order water-soluble complexes of the NSAID's and *N*-methylglucamine [11, 12].

For the arylacetic acid derivatives indomethacin and sulindac, slight non-linear decreases in solubility were observed above 0.15 M at pH 3.4. For example a linear increase in solubility of sulindac (y = 9.305 x – 0.1285) predicted a solubility of 1.74 mg/ml at 0.2 M *N*-methylglucamine. This was significantly higher than the measured solubility of 1.48 ± 0.084 mg/ml. According to Maurin et al. [13] some compounds form intermolecular hydrogen bonds when reacting with hydrotrope compounds (self-association). This reduces the ability of the drugs to interact with water and could explain the low solubility enhancement ratios for sulindac and indomethacin.

At pH 4.2 a non-linear decrease in solubility was observed above 0.15 M *N*-methylglucamine (Fig. 2). The apparent decrease in solubility enhancement at pH 4.2 might be attributed to the higher ionic strength in the buffer and the salt sensitivity of the NSAID's, where salting out might occur at high concentrations of *N*-methylglucamine. This effect was less pronounced for indomethacin and sulindac (Fig. 2).

The NSAID's tested contain at least one electronegative centre (the carboxylic group) and the interaction of *N*-methylglucamine with the drugs may be due to the interaction of a weak acid with the *N*-methylglucamine salt [1]. However, the solubility enhancement is remarkably high. As shown in Fig. 1 and 2 even at 0.025 M *N*-methylglucamine the solubility was significantly higher ($p < 0.05$). Therefore an increased solubility might further be attributed to some other phenomenon such as additional hydrogen bonding.

For all the NSAID's the increased solubility at low concentrations of *N*-methylglucamine suggested complexation and/or a weak interaction. Differences in the solubilisation behaviour and profiles (Figs. 1 and 2) of the *N*-methylglucamine complexes and salts of the different NSAID's could be attributed to self-association of the solubilisation products [13]. Shifts in λ_{max} of the drugs are indicative of such reactions; therefore the probability of interactions taking place between the drugs and *N*-methylglucamine was monitored by spectrophotometric measurements in the UV-range [1].

It was found that there was a change in the extinction coefficient of the drug in the presence of *N*-methylglucamine as a function of concentration. The extinction coefficients of the drugs decreased with increasing *N*-methylglucamine concentration. There were also slight shifts of 2–4 nm in the wavelengths of maximum absorbance of the drugs in the presence of *N*-methylglucamine.

Based on these results it can be concluded that the enhanced solubilisation of the NSAID's by *N*-methylglucamine can most probably be attributed to complexation between the drugs and the salt forming amine [13]. Complexation was afforded in a buffered system at low pH. The conversion of the NSAID's to the *N*-methylglucamine salts after complexation led to as much as two-fold increases in the solubilities of the NSAID's at very low concentrations of *N*-methylglucamine (0.0125 M). At 0.2 M the solubility increases measured were as much as 100 000 fold for ibuprofen at pH 4.2. This complexation and salt formation with *N*-methylglucamine may potentially provide alternatives for the development of higher concentration aqueous solutions of the NSAID's or improved dissolution of the NSAID's from oral solid dosage forms.

Experimental

1. Materials

N-Methylglucamine, ketoprofen, indomethacin, ibuprofen, naproxen and sulindac were at least 99% pure and were obtained from either Sigma Chemical Company or Fluka (Sigma-Aldrich, Atlasville, South Africa). Other chemicals were analytical grade. The solubilities were determined in sodium acetate, acetic acid buffers at pH 3.4 and 4.2 (2 M sodium acetate and 2 M acetic acid were used) containing increasing concentrations of *N*-methylglucamine from 0.0125 up to 0.2 M. The limited buffer capacity and the rise in pH with high *N*-methylglucamine concentrations restricted its use for studies at higher concentrations.

2. Solubility measurements

The solubility of the NSAID's in water was investigated according to the method of Higuchi and Lach [10]. An amount of drug enough to ensure saturation, 0.5 g, was suspended in 10 ml of the buffered solutions containing increasing amounts of *N*-methylglucamine (up to 0.2 M). The containers were rotated end-to-end for 48 h at 30 °C. Preliminary experimentation indicated that 48 h provided enough time to reach equilibrium. Experiments were done in triplicate. The suspensions were passed through a 0.45 μm filter (Osmonics, USA). The first part was discarded to ensure saturation of the filter. The concentrations of the dissolved drugs were determined at the wavelengths: $\lambda_m = 279$ nm for indomethacin; $\lambda_m = 286$ nm for sulindac; $\lambda_m = 262$ nm for ketoprofen; $\lambda_m = 263$ nm for naproxen; $\lambda_m = 265$ nm for ibuprofen [14].

3. Statistical analysis

Multivariate analysis of variance (MANOVA) including a post hoc comparison using the Newman-Keuls test was performed on the marginal means to look for significant differences (Statistica 5.1, StatSoft Inc., USA).

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Melgardt M. de Villiers, Ph.D.
Research Institute for Industrial Pharmacy
School of Pharmacy
Potchefstroom University for CHE
Potchefstroom 2520
South Africa
iifmmdv@puknet.puk.ac.za

Department of Biophysics and Chemical Physics[1], Comenius University Bratislava, Slovakia, Department of Biophysics[2], Al. I. Cuza University, Iasi, Romania, Department of Molecular Biology and Biophysics[3], ETH, Zürich, Switzerland, Department of Anesthesiology and Pain Management[4], Southwestern, Dallas, USA

The changes of capacitance relaxation of bilayer lipid membranes induced by chlorpromazine

T. Hianik[1], M. Fajkus[1], B. Tarus[2], D. F. Sargent[3], V. S. Markin[4] and D. F. Landers[4]

Chlorpromazine (CHP) belongs to the class of neuroleptics of low potency. Although both the existence of binding sites of CHP near the ion channel of the acetylcholine receptor [1] and the obtained activation of chloride currents in oocytes [2] might suggest a direct effect of CHP on membrane proteins, the indirect, lipid-mediated effect of CHP on biomembranes should not be excluded [3]. CHP is known to influence the shape of red blood cell membranes [4], and it can mediate the interaction of some drugs (e.g. *cis*-flupentixol) with phospholipids [5]. CHP decreases the membrane fluidity although it does not significantly influence the rotation relaxation time of 1,6-diphenyl 1,3,5-hexatriene (DPH) fluorescence probe.

In our recent work [6] we showed that a non-specific interaction of an amphiphilic drug – the local anesthetic tetracaine (TTC) – with bilayer lipid membranes (BLM) resulted in a more positive membrane surface potential. The surface potential increased with increasing concentration of TTC and was higher for the less charged from (at pH 9) than for the more highly charged one (pH 6). We proved that the main contribution to the change of surface potential comes from a dipole contribution. The 33 μM TTC concentration induced changes of dipole potential of about (5.3 ± 2.0) mV and (29.8 ± 3.0) mV at pH 6 and pH 9 respectively, which corresponds to a change of surface dipole moment of 8.1 ± 3.0 and 34.0 ± 3.5 Debye, respectively. Unmodified BLMs were characterized by a single relaxation time of about 5 μs that correspond to reorientation of individual molecular dipoles. Addition of TTC (final concentration 0.1 mM) resulted in the appearance of an additional, slower relaxation component ($\tau = 50$ μs) at electrolyte pH 9, while at pH 6 no changes of relaxation time occurred. We assumed that due to its more neutral form at pH 9 TTC could penetrate more deeply into the lipid bilayer. Interaction of TTC with BLM probably induces heterogeneity of phospholipid environment and perturbs the bilayer dynamics. CHP, like TTC, is an amphiphilic molecule, and the drugs have similar ionization constants (pK_a 9.3 for CHP and pK_a 9.5 for TTC). While the polar parts of TTC and CHP are similar, the differences in the structure of the non polar parts might result in different abilities of their neutral forms to penetrate into the membrane and thus might result in dif-

Table: Relaxation times (τ, μs) of reorientation of molecular dipoles in polar region of BLM of egg PC + cholesterol with and without chlorpromazine (CHP) (final concentration 0.1 mM)

Egg PC + cholesterol		Egg PC + cholesterol + CHP	
pH 5.5	pH 9.5	pH 5.5	pH 9.5
4.5 ± 0.31	4.1 ± 0.12	4.83 ± 0.14 8.16 ± 0.63	4.47 ± 0.31

Means ±S.D. were obtained by averaging of 64 current relaxation curves from one