Supplementary

Regression analysis data, determination of ionization constant, and preparation of diclofenac base

Abdussalam A.M. Amara[1*], Michael B. Lambert[2], Patrick B. Deasy[3]

[1]Department of Pharmaceutics, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya, [2]Department of Pharmacology and Therapeutics, [3]Department of Pharmaceutics, School of Pharmacy, Trinity College, University of Dublin, Dublin, Ireland.

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*Corresponding author: draomara@yahoo.com

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Regression analysis data for the assay calibration curves for NSAIDs studied

In purified water				
Drug	**N**	**R**	**s**	**best fit equation**
Biphenylacetic Acid	6	0.992	0.071	A = 0.105 + 0.027 C
Diclofenac	6	0.992	0.015	A = 0.021 + 0.006 C
Diclofenac Na	6	0.996	0.044	A = 0.066 + 0.017 C
Indomethacin	6	0.991	0.028	A = 0.041 + 0.013 C
Piroxicam	6	0.993	0.075	A = 0.111 + 0.028 C
In phosphate buffer (*p*H 7.4)				
Drug	**N**	**r**	**s**	**best fit equation**
Biphenylacetic Acid	6	0.999	0.014	A = 0.004 + 0.021 C
Diclofenac	6	1.000	0.021	A = 0.018 + 0.039 C
Diclofenac Na	6	1.000	0.012	A = 0.003 + 0.029 C
Indomethacin	6	0.992	0.094	A = 0.141 + 0.036 C
Piroxicam	6	0.991	0.101	A = 0.147 + 0.038 C
In *n*-octanol				
Drug	**N**	**R**	**s**	**best fit equation**
Biphenylacetic Acid	6	1.000	0.016	A = 0.008 + 0.051 C
Diclofenac	6	0.998	0.029	A = 0.016 + 0.037 C
Diclofenac Na	6	1.000	0.011	A = 0.008 + 0.041 C
Indomethacin	6	0.966	0.031	A = 0.001 + 0.015 C
Piroxicam	6	0.999	0.013	A = 0.002 + 0.048 C

where, n = number of observations, r = regression coefficient, s = standard error, A = absorbance, and C = concentration of the compound in μg ml^{-1}.

Determination of ionization constant (pk_a)

pk_a determination of biphenylacetic acid					
***p*H**	**A**	**$A - A_i$**	**$A_m - A$**	$log\frac{A-A_i}{A_m-A}$	**pk_a**
4.75	0.602	0.213	1.313	-0.790	**3.96**
4.55	0.725	0.336	1.190	-0.549	**4.00**
4.35	0.842	0.453	1.073	-0.375	**3.98**
4.15	0.996	0.577	0.949	-0.216	**3.93**
3.95	1.205	0.816	0.710	0.060	**4.01**
3.75	1.325	0.936	0.590	0.200	**3.95**
3.55	1.445	1.056	0.470	0.352	**3.90**
pk_a values (mean ± SD, at 21°C)					**3.96 ± 0.04**

pk_a determination of diclofenac sodium					
***p*H**	**A**	**$A - A_i$**	**$A_m - A$**	$log\frac{A-A_i}{A_m-A}$	**pk_a**
5.97	0.898	0.669	0.831	-0.090	5.84
5.77	1.014	0.785	0.715	0.410	5.77
5.57	1.111	0.882	0.618	0.155	5.96
5.37	1.293	1.064	0.436	0.369	5.70
5.13	1.410	1.181	0.319	0.569	5.70
4.97	1.489	1.260	0.240	0.720	5.65
4.73	1.553	1.324	0.176	0.876	5.61
pk_a values (mean ± SD, at 21°C)					**5.71 ± 0.08**

pk_a determination of diclofenac					
***p*H**	**A**	**$A - A_i$**	**$A_m - A$**	$log\frac{A-A_i}{A_m-A}$	**pk_a**
5.54	0.616	0.424	0.940	-0.346	**5.19**
5.34	0.751	0.559	0.805	-0.158	**5.18**
5.14	0.875	0.683	0.681	0.001	**5.14**
4.94	1.015	0.823	0.541	0.182	**5.12**
4.74	1.112	0.920	0.444	0.316	**5.06**
4.54	1.237	1.045	0.319	0.515	**5.06**
4.34	1.343	1.151	0.213	0.733	**5.07**
pk_a values (mean ± SD, at 21°C)					**5.12 ± 0.06**

pk_a determination of indomethacin					
***p*H**	**A**	**$A - A_i$**	**$A_m - A$**	$log\frac{A-A_i}{A_m-A}$	**pk_a**
5.41	0.310	0.141	0.782	-0.744	**4.67**
5.21	0.383	0.211	0.712	-0.528	**4.68**
5.01	0.461	0.289	0.634	-0.341	**4.67**
4.81	0.523	0.351	0.572	-0.212	**4.60**
4.61	0.637	0.465	0.458	0.007	**4.62**
4.41	0.759	0.567	0.336	0.242	**4.65**
4.21	0.871	0..699	0.224	0.494	**4.70**
pk_a values (mean ± SD, at 21°C)					**4.66 ± 0.04**

pk_a determination of piroxicam					
pH	**A**	**$A - A_i$**	**$A_m - A$**	$log \frac{A - A_i}{A_m - A}$	**pk_a**
4.84	0.727	0.038	0.258	-0.831	6.01
6.64	0.753	0.064	0.232	-0.559	6.08
6.44	0.787	0.098	0.198	-0.305	6.14
6.24	0.821	0.132	0.164	-0.094	6.15
6.04	0.851	0.162	0.134	0.082	6.12
5.84	0.886	0.197	0.099	0.299	6.14
5.64	0.912	0.223	0.073	0.485	6.13
pk_a values (mean ± SD, at 21°C)				**6.11 ± 0.05**	

Preparation of diclofenac base

Diclofenac was prepared from diclofenac sodium as follows: diclofenac sodium was dissolved in the minimum amount of methanol (i.e. 1 in 10 ml) in a 50-ml beaker and about 5 ml of concentrated hydrochloric acid was added followed by the addition of 40 ml of purified water and was transferred to a 1000-ml separating funnel. The beaker was then washed with 2 x 20 ml of purified water and added to the separating funnel to bring the volume of water to 80 ml. finally, 2 x 50 ml of ether was added to the separating funnel. This system was well shaken for 10 minutes and allowed to separate into two phases, the ether phase on the top containing the base and the water phase being on the bottom containing the sodium. The lower phase was discarded and the upper phase was transferred into a flask. The ether phase in the flask was dried with magnesium sulfate ($MgSO_4$) overnight at room temperature. The ether was then evaporated using a rotary evaporator, and diclofenac was then collected. In order to confirm the conversion of diclofenac sodium to diclofenac base, further comparison of structural analysis on both agents was carried out by infra-red (IR) spectroscopy and nuclear magnetic resonance (NMR). Diclofenac sodium spectrum (**Fig. 1B**) showed principle peaks at wavenumbers 3378, 3258 and 1576, 1557 cm^{-1} frequencies for NH and CO stretching, respectively. Both peaks showed clearly a split due to the effect of the sodium metal that is present in this compound. However, in the case of diclofenac base (**Fig. 1A**), the IR spectroscopy showed that the NH stretching frequency at 3323 cm^{-1} and the CO stretching frequency at 1695 cm^{-1} as single sharp peaks with no splitting, indicating that no sodium is present in the compound. In addition, a broad peak at 2692 cm^{-1} frequency is seen in the spectrum which is assigned for the OH stretching. The IR spectra indicate that the ONa group was substituted by OH group. In order to know whether the conversion of diclofenac sodium to diclofenac base has really occurred, further comparison of structural analysis on both drugs was carried out by NMR (**Figure 2**). The results show that proton (1H) consists of a doublet δ7.52 ppm assigned to $H_{3,5}$ (2H), a triplet at δ7.17 ppm assigned to H_4 (1H), a doublet at δ7.24 ppm due to H_9 (1H), a triplet at δ6.88 ppm to H_{10} (1H), a triplet at δ7.08 ppm assigned to H_{11} (1H), a doublet δ6.33 ppm assigned to H_{12} (1H), and two singlets at δ7.30 ppm and δ3.75 ppm assigned to NH (1H) and CH_2 (1H), respectively. The ^1H NMR spectrum for diclofenac base (**Fig. 2**) showed the aromatic protons as a doublet at δ7.44 ppm assigned to $H_{3,5}$ (2H), a triplet at δ7.07 ppm assigned to H_4 (1H), a doublet at δ7.15 ppm due to H_9 (1H), a triplet at δ6.77 ppm to H_{10} (1H), a triplet at δ6.95 ppm assigned to H_{11} (1H), a doublet δ6.30 ppm assigned to H_{12} (1H), and three singlets at δ9.96, δ3.82 and δ3.75 ppm and δ3.75 ppm assigned to NH (1H) and CH_2 (2H) and OH (1H), respectively, therefore, diclofenac showed the presence of the OH signal.

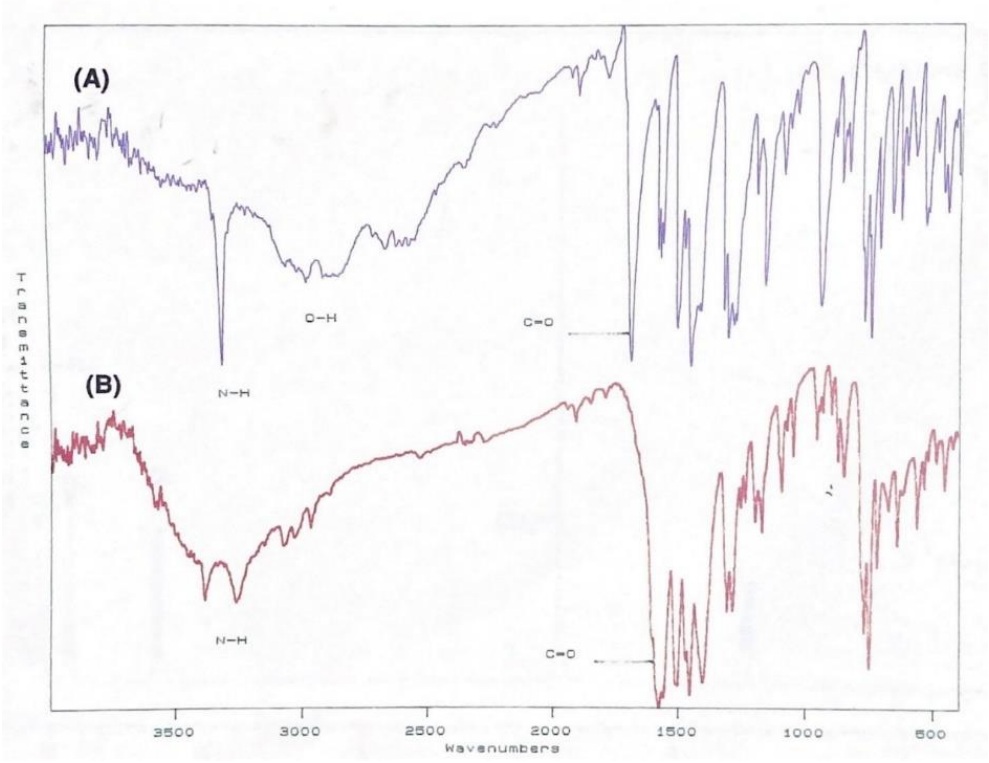
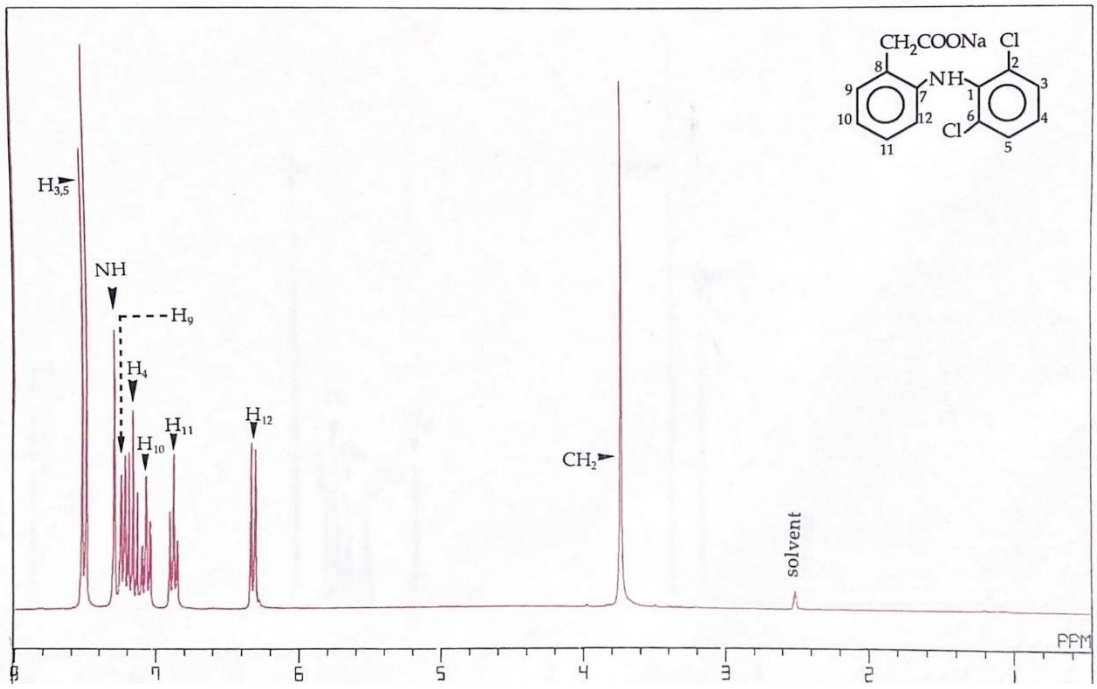


Figure (1): IR spectra for diclofenac base (A) and diclofenac sodium (B).

Diclofenac sodium



Diclofenac base

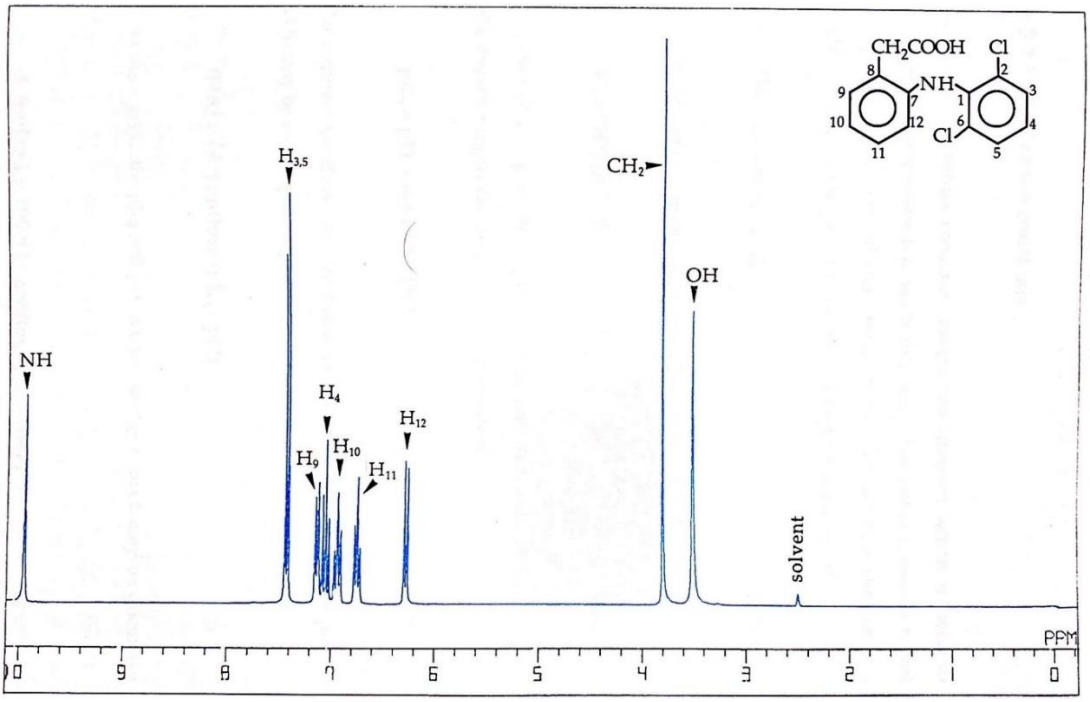


Figure (2): Proton NMR spectrum for diclofenac sodium and diclofenac base.