EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
17-22
Year: 2018

PHYSICOCHEMICAL CHARACTERIZATION OF BUDESONIDE FOR NANOPARTICLES PREPARATION.

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Article Received on 24/12/2017 **Article Revised on 14/01/2018** **Article Accepted on 04/02/2018**

ABSTRACT
Preformulation studies and characterization are known as a special fundamental Physico-chemical properties of the drug such as drug solubility, stability, melting point, and drug partitioning. Preformulation studies can help in selecting formulation approach to develop effective, stable, and economic dosage forms. Fundamental Preformulation studies include development of a suitable analytical method for drug analysis, determination of drug solubility, determination of drug stability at different stress conditions which may be applied during the preparation of formulations, determination of water/octanol partition coefficient, measurement of melting point and drug excipients compatibility. Physicochemical properties of budesonide such as aqueous solubility in deionized water, different buffer solutions, and solutions containing surfactants were determined. Partition coefficient using water/ octanol system at different pH values were calculated.Forced degradation studies under different stress conditions such as acid, alkali, photo, oxidative, and neutral conditions were studied using LC-MS-MS spectrophotometer. Budesonide showed aqueous solubility of 0.02566 mg/mL with increased solubility to some extent upon using tween 80. Budesonide have a partition coefficient of 2.14 with good drug stability under different stress conditions except higher alkali conditions in which drug completely degraded into another product. Physicochemical studies for the poorly soluble budesonide offered a suitable data for the preparation of nanoparticles.

KEYWORDS: Budesonide, aqueous solubility, partition coefficient, forced degradation studies.

INTRODUCTION
Budesonide is a potent, non-halogenated glucocorticoid, used in the treatment and management of asthma, COPD, skin disorders as allergies, allergic rhinitis and inflammatory bowel disease[1], specifically for left-sided colitis. The drug was approved by FDA for treatment and management of ulcerative colitis on January 14, 2013. Budesonide is supplied as a mixture of 22 R and 22 S epimers. Budesonide has a high ratio of topical to systemic activity compared to the reference corticosteroids as beclomethasone dipropionate. Administration of budesonide includes inhalers for the management of asthma and enema and extended-release tablets or capsules for the management of ulcerative colitis.[2] Budesonide is a mixture of two epimers of the α-and β-propyl forms of 16α, 17α-butylidenedioxy-11β, 21-dihydroxypregna-1, 4-diene-3, 20-Dione. Chemically named (11beta, 16alpha)-16, 17-(Butylidenebis(oxy))-11,21-dihydroxypregna-1,4-diene-3,20-dione. It has empirical formula $C_{25}H_{34}O_6$ and molecular weight of 430.5 g/mol. The melting point of the drug is about 224°C (224-226°C). Budesonide is white fine powder; it is practically insoluble in water, freely soluble in chloroform and dichloromethane, sparingly soluble in alcohols and soluble in acetone. The drug has a partition coefficient (Log P) of 1.9-2.4 (calculated values) and has pKa value of strongest acidic 13.75. Budesonide is stable under normal storage conditions, kept in tightly closed containers at a temperature of 20-25°C and protect from light. (**Fig. 1**) show budesonide structure.[2,3] Drug solubility were carried out in deionized water and at different pH values, partition coefficient was determined at different pH values. From the other hand, budesonide was exposed to different stress conditions including acid, alkali, photo, thermal and oxidative stress conditions.

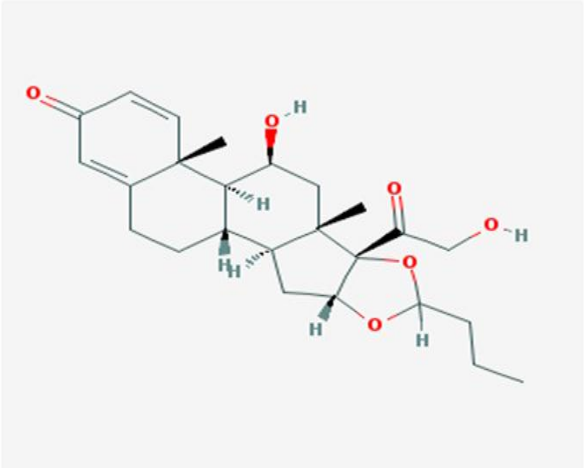

Fig. 1: Chemical structure of budesonide.

1. MATERIALS AND METHODS

1.1. Materials

Budesonide (obtained as a gift sample from MUP Company, Egypt.). Deionized water (Milli-Q, obtained from water analysis Lab., Minia, Egypt). HPLC grade methanol (was purchased from sigma Aldrich chemicals Co.). Octanol (purchased from Sigma Aldrich chemicals Co.). Tween 80 (Purchased from sigma Aldrich chemicals Co.). All other solvents and buffer salts were of analytical grade.

1.2. Methods

1.2.1. Determination of budesonide aqueous solubility

Equilibrium solubility studies of BSD were performed in deionized water, 0.1 N HCL solution pH 1.2 was prepared as US pharmacopeia (0.1 N hydrochloric acid HCL, 2 g sodium chloride NaCl, 1L distilled water), phosphate buffer pH 6.8 solution (potassium dihydrogen orthophosphate KH2PO4 (6.8 g), sodium hydroxide NaOH (0.94 g), 1L distilled water), phosphate buffer solution (PBS) of pH 7.4 (NaCl 8 g, disodium phosphate Na_2HPO_4 2.38g, potassium dihydrogen orthophosphate KH_2PO_4 0.19g, distilled water 1L) and the same solutions but containing tween 80 (0.2% W/V) as solubility enhancer, at 37ºC ± 0.5ºC. The pH was adjusted to the desired value using 1 M HCL and 1 M NaOH. An excess amount of the BSD was dispersed in 5-mL of different prepared solutions of different pH in 10-mL screw-capped tubes and shacked at 100 strokes/min using a thermally-controlled shaking water bath for 48 h and left for another 24 h without shaking to achieve equilibrium solubility. The samples were centrifuged at 5000 rpm for 15 min at 25ºC and then filtered through 0.22 µm filter membrane. Samples measured spectrophotometrically at 244 nm.

1.2.2. Determination of n-octanol/ buffer solutions partition coefficient

The distribution of BSD between equal volumes of two phases n-octanol (oil phase) and previously prepared buffer solutions (aqueous phase) was determined at different pH values (1.2, 6.8 and 7.4) at 37ºC ± 0.5ºC, the pH of different buffers adjusted to required values. Both n-octanol and buffer solutions were vortexed for 2 min and mutually saturated overnight at least for 12 h at the corresponding temperature before use. A specified amount of BSD was dissolved in presaturated n-octanol (oil phase) with buffer solutions (aqueous phase). Aliquots of 2-mL of BSD solution was mixed with equal volumes of buffer solution in screw-capped glass vials and vortexed for 2 min. then the glass vials were kept for a period of 12 h at the corresponding temperature using thermally-controlled water bath which was sufficient to allow the drug to reach distribution equilibrium between the two phases. After the equilibrium a micropipette was used to separate two phases, followed by centrifugation (5000 rpm for 15 min at 25ºC) to remove any of the remaining immiscible n-octanol phase from the aqueous phase. Aliquots of both aqueous and oil phases were taken and the concentration in both buffer solutions and n-octanol phase were measured spectrophotometrically at 244 nm.[4]

1.2.3. Forced degradation studies

1.2.3.1. Acid degradation

For acidic degradation of BSD, 10-mL of BSD stock solution (methanol solution) with a final concentration of 100 µg/mL was transferred to 20-mL amber-colored volumetric flask and volume was made up to 20 mL with 1 M hydrochloric acid (HCL). The flask was tightly closed and kept at 85ºC for 2 h using thermally-controlled water bath, then the flask was cooled to room temperature, then neutralized using 1 M NaOH and volume was readjusted using methanol solution, the solution was filtered using 0.22 µm filter membrane and injected to LC-MS-MS to detect degradation peaks qualitatively and quantitatively.[5]

1.2.3.2. Alkali degradation

For basic degradation of BSD, 10-mL of BSD stock solution (methanol solution) with a final concentration of 100 µg/mL was transferred to 20-mL amber-colored volumetric flask and volume was made up to 20 mL with 1 M sodium hydroxide (NaOH). The flask was tightly closed and kept at 85ºC for 2 h using thermally-controlled water bath, then the flask was cooled to room temperature, then neutralized using 1 M HCL and volume was readjusted using methanol solution, the solution was filtered using 0.22 µm filter membrane and injected to LC-MS-MS to detect degradation peaks qualitatively and quantitatively.[5]

1.2.3.3. Thermal degradation

For thermal degradation of BSD, 10-mL of BSD stock solution (methanol solution) with a final concentration of 100 µg/mL was transferred to 20-mL amber-colored volumetric flask and volume was made up to 20 mL with the mobile phase, The flask was tightly closed and kept at 85ºC for 2 h using thermally-controlled water bath, then the flask was cooled to room temperature, then the volume was readjusted using the mobile phase solution,

the solution was filtered using 0.22 µm filter membrane and injected to LC-MS-MS to detect degradation peaks qualitatively and quantitatively.[5]

1.2.3.4. Neutral degradation

For neutral degradation of BSD, 10-mL of BSD stock solution (methanol solution) with a final concentration of 100 µg/mL was transferred to 20-mL amber-colored volumetric flask and volume was made up to 20 mL with the deionized water, The flask was tightly closed and kept at 85ºC for 2 h using thermally-controlled water bath, then the flask was cooled to room temperature, then the volume was readjusted using the methanol solution, the solution was filtered using 0.22 µm filter membrane and injected to LC-MS-MS to detect degradation peaks qualitatively and quantitatively.

1.2.3.5. Oxidative degradation

For oxidative degradation of BSD, 10-mL of BSD stock solution (methanol solution) with a final concentration of 100 µg/mL was transferred to 20-mL amber-colored volumetric flask and volume was made up to 20 mL with 5% hydrogen peroxide (H_2O_2), The flask was tightly closed and kept at 85ºC for 2 h using thermally-controlled water bath, then the flask was cooled to room temperature, then the volume was readjusted using methanol solution, the solution was filtered using 0.22 µm filter membrane and injected to LC-MS-MS to detect degradation peaks qualitatively and quantitatively.[5]

2. RESULTS AND DISCUSSION

2.1. Aqueous solubility of budesonide

Determination of aqueous solubility of budesonide at different pH values was carried out, BSD is practically insoluble in water even in different pH and for example, the observed solubility of the drug in deionized water is 0.02566±0.00614 mg/mL which is near to the reported value in literature (28 µg/mL)[6] and 21.5 µg/mL[7], when the pH changed to 1.2 solubility of the drug approximately as the same as solubility in deionized water, in the same way, increasing pH to 6.8 the amount of drug solubilized increased insignificantly (P>0.05) to 0.046225±0.001 mg/mL. Also when the pH changed to 7.4, the drug solubility increased insignificantly (P>0.05) to 0.0669±0.0126 mg/mL. The following solubility data indicates that the drug is practically insoluble in water and pH change have no effect on the drug solubility as the drug solubility depends mainly on partition coefficient. From the previous data Log S = -4.2 (logarithm of solubility in mole/litter) which confirmed by many calculated data such as, budesonide have Log S = -4.73 (ChemAxon) and -4 (ALOGPS). On the other hand, from the calculated solubility using *Yalkowsky and Valvani* equation.[8]

$$\log Sw = -\log Pc - 0.012tm + 0.87$$ Equation 1.

Where, (S_w) is the drug solubility, (P_c) is the octanol/ water partition coefficient and (t_m)is the melting point of the drug. From the above equation Log S = -4.3. Also, by using of *Jain and Yalkowsky* equation[8], in which Log S = -4.5.

$$\log Sw = -1.031 \log Pc - 0.01202tm + 0.679$$ Equation 2.

(**Table 1**) shows the observed aqueous solubility data compared with calculated and predicted data. From another point of view, solubility data of budesonide was obtained using tween 80 at a concentration of (0.2%, W/V) in deionized water, buffer pH 1.2, 6.8 and 7.4. Tween 80 significantly (P value = 0.0004, 0.0004, 0.0009 and 0.0001) increase drug solubility by decreasing surface tension, increasing drug wettability and entrapping of lipophilic drugs within the formed micelles[9] (tween in this case used at a concentration above its critical micelle concentration, CMC 0.0016% W/V). (**Table 2**) shows solubility data of BSD in different pH containing tween 80 (0.2% W/V).

Table 1: Aqueous solubility data at different pH.

Media	Solubility (mg/mL)	Log S [a]
		Observed
Deionized water	0.02566±0.00614	- 4.2
Media with pH 1.2	0.02575±0.00543	- 4.2
Buffer with pH 6.8	0.04622±0.00104	- 3.9
Buffer with pH 7.4	0.06690±0.01268	- 3.8

[a]product of Log S is the solubility in Mole/L unit.

Table 2: Solubility data of BSD in different buffers containing tween 80 (0.2% W/V).

No.	Media	Solubility*	
		Solubility (mg/mL)	Log S
1	Deionized water	0.193599±0.014566	-3.34
2	Media with pH 1.2	0.193599±0.014566	-3.34
3	PBS buffer pH 6.8	0.176449±0.010450	-3.38
4	PBS buffer pH 7.4	0.376449±0.014232	-3.05

***** Solubility data presented as mean solubility ± SD.

2.2. Determination of partition coefficient

Distribution coefficient (D) is the ratio between drug concentration in the oil phase (unionized form) and drug concentration in the aqueous phase (both unionized and ionized species). The true partition coefficient (P) is the ratio between the concentration of unionized form in the oil phase (n-octanol) and concentration of unionized form in the aqueous phase (water).[10] In this study, aqueous phase is buffered with different pH values, 1.2, 6.8 and 7.4 which represents gastrointestinal pH values on the stomach, lower intestine and upper intestine (ileum). The distribution coefficient is determined at 37°c (temperature of the human body). Log P values from 1 to 3 indicates that the drug is lipophilic and with good absorption.[11]

The calculated values of distribution coefficient (D) in different pH values were converted through equation 1.2 to the true value of partition coefficient (P). All experiments are prepared in three independent preparation, mean value was calculated ± SD. The calculated D values showed an insignificant change in results with pH changes which indicate that the drug is lipophilic and its aqueous solubility in different pH values is pH-independent. The results of the partition coefficient (P) is confirmed by previously determined solubility data which indicates that the drug solubility has insignificantly changed by changing pH value.

The Calculated (P) values were compared to predicted values in (ChemAxon), (ALOGPS) and with experimentally determined data in the literature. (**Table 3 and 4**) shows experimentally calculated data while the later shows the calculated data compared with predicted values at pH 7.4.

Table 3: Experimentally calculated D app, Log P values at different pH values. Results are expressed as mean value ± SD.

pH	D (app)	pKa*	Log P (D app)	Log P
1.2	103.6 ± 26.228	13.75	2.01549 ± 0.1167	2.01549 ± 0.1167¤
6.8	278.2 ± 44.368	13.75	2.44447 ± 0.0689	2.44447 ± 0.0689¤
7.4	139 ± 4.978	13.75	2.14332 ± 0.0157	2.14332 ± 0.0157¤

* predicted from ChemAxon, also reported in literature from 12-14[12], strongest acidic pKa.
¤*P* value = 0.0500 is not quite significant.

Table 4: Calculated log P of BSD compared with both predicted and observed log P from previous literature at pH 7.4.

pH	Calculated Log P	Predicted Log P value*			From literature		
		Chem Axon[13]	ALOGPS[14]	HMDB§[15]	Ref.[16]	Ref.[17]	Ref.[11]
7.4	2.1	2.73	2.42	1.9	3.2	3.21	2.6 ¤

* Predicted using of online software.
§ HMBD, human metabolome data base.
¤ According to method of determination.

2.3. Forced degradation studies

LC-MS-MS spectrum showed no degradation peaks observed for budesonide under acid, thermal, neutral and oxidative stress conditions (**fig. 2**). On the other hand, mass spectrum under alkali stress conditions showed that budesonide totally degraded in alkali especially at higher pH level (**fig. 3**). Degradation product of budesonide cannot determined using UV-VIS spectrophotometer as the degradation product have the same wave length maxima of budesonide at 244 nm (**fig. 4**) as both compounds containing intact Enone chromophore.[5]

Alkali degradation is due to the presence of acidic protons at C_{21} which under strongly alkaline conditions led to cleavage of ring E, loss of propyl function group (C_{22}-C_{25}) and finally cyclization produce degradation product with molecular weight about 342 g/mole with loss about 88 molecular weight. The new compound is confirmed from the previously reported literature.[5]

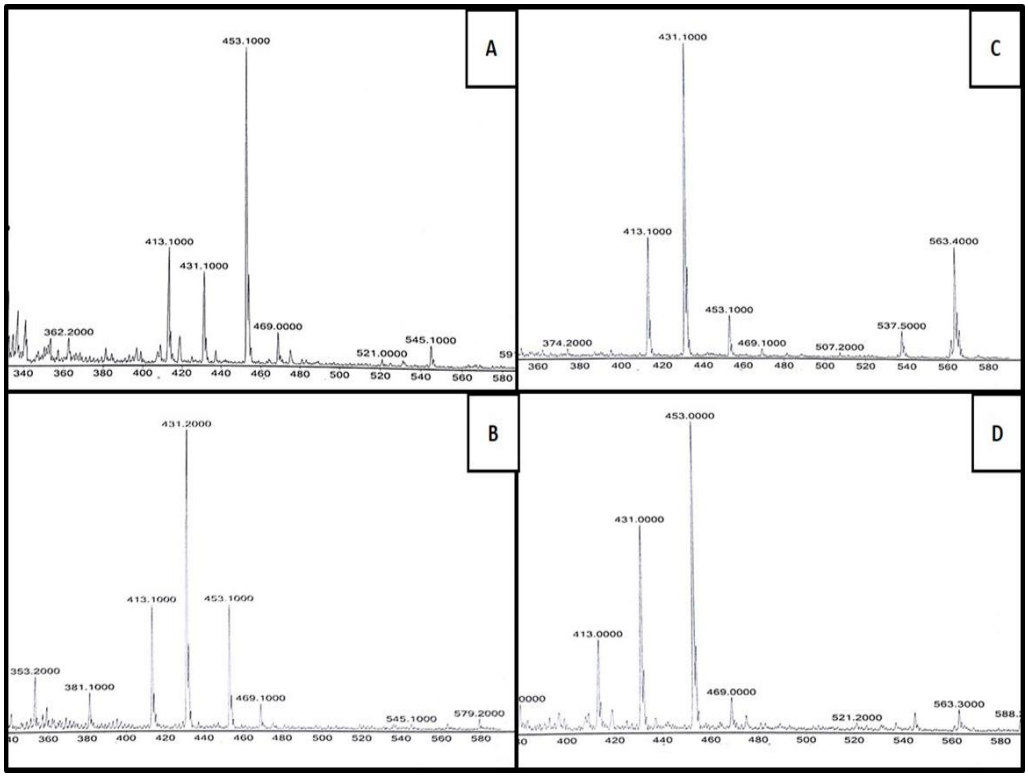

Fig. 2: LC-MS-MS spectrum of budesonide under neutral stress conditions (A), thermal stress conditions (B), acidic stress condition (C) and oxidative stress condition (D).

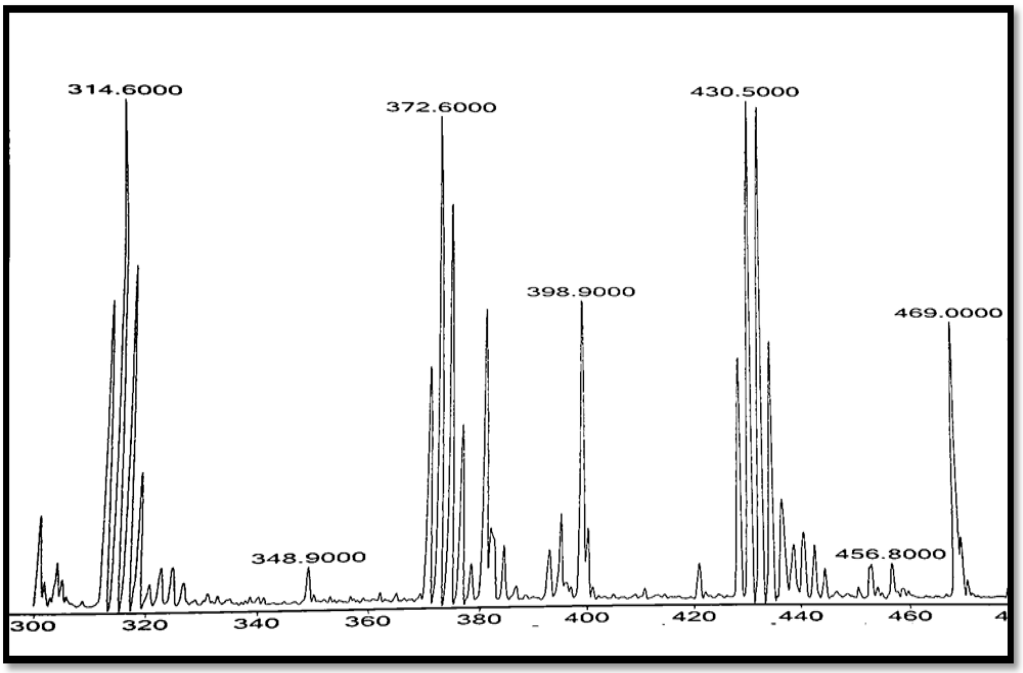

Fig. 3: LC-MS-MS spectrum shows degradation product of budesonide under alkali stress conditions.

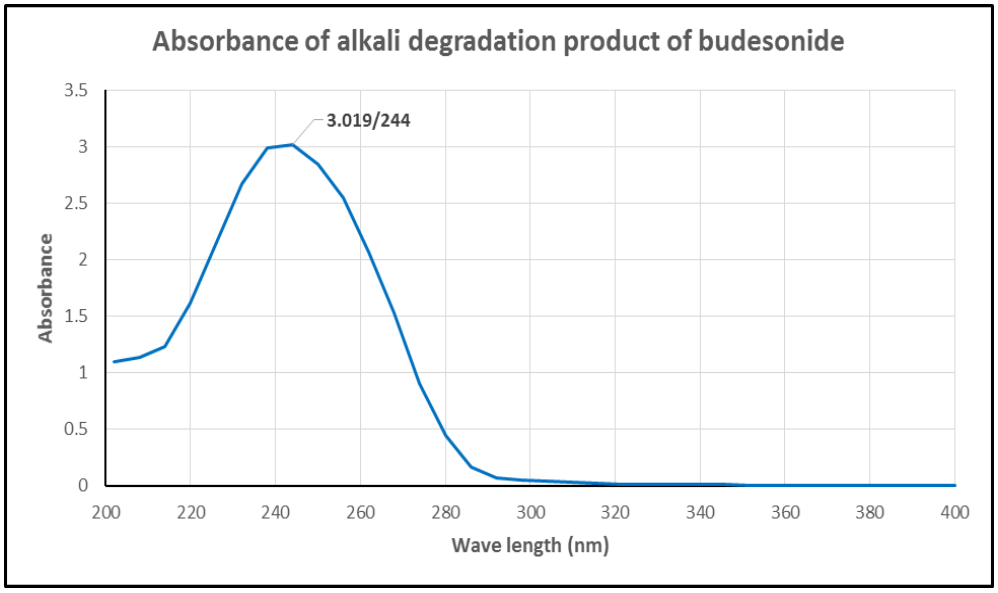

Fig. 4: Absorbance of alkali degradation product of budesonide.

3. CONCLUSION

Budesonide is a potent glucocorticoid used for the treatment of various inflammatory conditions. Budesonide is characterized from physicochemical point by the lipophilic nature which reflected by low aqueous solubility data of 25 µg/mL and higher partition coefficient (log P) of 2.4. Budesonide is stable under acid, neutral, photo, thermal stress conditions, but unstable under higher alkali conditions.

4. ACKNOLWEDGMENT

Great thanks to MUP Company, Egypt for generously providing gift budesonide samples.

5. CONFLICT OF INTREST

All the authors of the manuscript do not have any conflict of interest.

6. REFERENCES

1. Prabhu, P., et al., *Investigation and comparison of colon specificity of novel polymer khaya gum with guar gum.* Pakistan journal of pharmaceutical sciences, 2010.
2. Sweetman, S.C., *Martindale: the complete drug reference*. 2009: Pharmaceutical press.
3. Pharmacopoeia, B., *British Pharmacopoeia Commission London; the Department of Health.* Social Services and Public Safety, 2013; 1: 719-720.
4. Florence, A. and D. Attwood, *The solubility of drugs*, in *Physicochemical Principles of Pharmacy*. 1998, Springer. p. 152-198.
5. Naikwade, S.R. and A.N. Bajaj, *Development of validated specific HPLC method for budesonide and characterization of its alkali degradation product.* Can J Anal Sci Spect, 2008; 53: 113-122.
6. Ali, H.S., et al., *Solubility of budesonide, hydrocortisone, and prednisolone in ethanol+ water mixtures at 298.2 K.* Journal of Chemical & Engineering Data, 2009; 55(1): 578-582.
7. Yalkowsky, S.H., Y. He and P. Jain, *Handbook of aqueous solubility data*. 2016: CRC press.
8. Ellenberger, D., K.P. O'Donnell and R.O. Williams III, *Optimizing the Formulation of Poorly Water-Soluble Drugs*, in *Formulating Poorly Water Soluble Drugs*. 2016, Springer. p. 41-120.
9. Savjani, K.T., A.K. Gajjar, and J.K. Savjani, *Drug solubility: importance and enhancement techniques.* ISRN pharmaceutics, 2012. 2012.
10. Sangster, J., *Octanol-water partition coefficients of simple organic compounds.* Journal of Physical and Chemical Reference Data, 1989; 18(3): 1111-1229.
11. S Bharate, S., V. Kumar and R. A Vishwakarma, *Determining partition coefficient (Log P), distribution coefficient (Log D) and ionization constant (pKa) in early drug discovery.* Combinatorial chemistry & high throughput screening, 2016; 19(6): 461-469.
12. Corey, E.J. and E.T. Fossel, *Transdermal formulations of fluticasone*. 2014, Google Patents.
13. Sari, T., et al., *Preparation and characterization of nanoemulsion encapsulating curcumin.* Food Hydrocolloids, 2015; 43: 540-546.
14. Schultze, E., et al., *Encapsulation in lipid-core nanocapsules overcomes lung cancer cell resistance to tretinoin.* European Journal of Pharmaceutics and Biopharmaceutics, 2014; 87(1): 55-63.
15. Selzer, D., et al., *Finite and infinite dosing: difficulties in measurements, evaluations and predictions.* Advanced drug delivery reviews, 2013; 65(2): 278-294.
16. Dörwald, F.Z., *Lead optimization for medicinal chemists: pharmacokinetic properties of functional groups and organic compounds*. 2012: John Wiley & Sons.
17. Lin, H., et al., *Transport of anti-allergic drugs across the passage cultured human nasal epithelial cell monolayer.* European journal of pharmaceutical sciences, 2005; 26(2): 203-210.