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Analysis of methotrexate in pharmaceutical formulations by UV-Visible spectrophotometric method

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ABSTRACT

A simple, sensitive, selective rapid spectrophotometric method has been developed for the determination of post synaptic α_1- Adrino receptor antagonist Methotrexate in pure form and pharmaceutical formulations based on the oxidative coupling reaction with 2, 2- Bipyridine reagent, at P^H–4.0 which is extractable at 510 nm. Beer's law is obeyed in the concentration ranges 4-24 µg ml^{-1}. The developed method was applied directly and easily for the analysis of the Pharmaceutical formulations. R.S.D was found to be 0.2859% and Recovery 98.99% respectively. The method was completely validated and proven to be rugged. The interferences of the other ingredients and excipients were not observed. The repeatability and the performance of the proved method were established by point and internal hypothesis and through recovery studies.

Keywords: Spectrophotometry, Methotrexate 2, 2- Bipyridine / $FeCl_3$

INTRODUCTION

Methotrexate-{(2S)-2-[(4-{[(2,-diaminopteridin-6-1(methyl)](methyl)amino}phenyl)formamide]}is a Antineoplastic anti-metabolite. Methotrexate anti-tumor activity is a result of the inhibition of folic acid reductase, leading to inhibition of DNA synthesis and inhibition of cellular replication. It is structurally related to prazosin (Figure1)

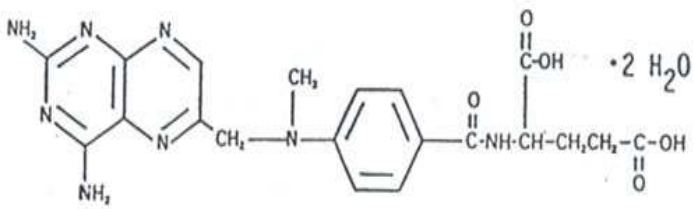


Figure.1

A survey of the literature revealed that different analytical techniques for the assay of MTX have been reported. HPLC with fluorimetric[4-13] and UV[14-19] detection methods have been reported. Using the former methods, the derivatization reactions include; photo-oxidative irradiation at 510 nm in the presence of hydrogen peroxide to yield 2,4-diaminopteridine-6-carboxylic acid[6,11-13], oxidation with permanganate in presence of acetate buffer (pH 4.0) to give 2-amino-4-hydroxypteridine-6-carboxylic acid[10], and oxidative cleavage using either phosphate buffer

containing 0.2 of 30% hydrogen peroxide[4,8&9] or pre-column of cerium (IV) trihydroxyhydroperoxide[5,7] to get 2,4-diaminopteridine-6-carboxylic acid. The latter reaction has been applied in flow injection technique[20].

Early, spectrofluorimetric methods based on oxidation of MTX to pteridine carboxylic acid, using permanganate, have been described[21&22]. The latter has been applied to study plasma levels in cancer patients with a limit of determination of 100 mg ml^{-1}. Spectrophotometric methods including color reactions[23&24] and UV measurements[25] have been described. Polarographic and voltammetric methods[26-29] for the quantitation of MTX in pharmaceuticals and plasma samples have been published.

The main subject of this article is the spectrofluorimetric determination of MTX through acid-catalyzed degradation reaction. The latter is based on the hydrolysis across the amide linkage. The previously described fluorimetric derivatization[4-13&21, 22] has concerned with the oxidative cleavage at the Methylamino Bridge. The present work was channeled through three approaches. First, to study the fluorescence and UV–Vis spectral characteristics of MTX and its acid-degradation product, AMP. Second, to investigate the kinetics of the degradation reaction of MTX in solutions of different acid-strengths. Nanonization of methotrexate by solution-enhanced dispersion by supercritical CO_2 Ai-Zheng Chen.

There is however no reported UV- Visible spectrophotometric method for the analysis of Methotrexate in its technical grade and formulations. UV- visible spectrophotometric method for the quantitative determination of Methotrexate. Functional group used for color development of Methotrexate was primary amine group. The results obtained in this method was based on complex formation reaction of Methotrexate with Oxidative coupling reaction with 2, 2- Bipyridine.

In the present study an attempt has been made to develop simple UV-Visible spectrophotometric method for quantitative estimation of Methotrexate in its technical grade, formulations and biological sample (Blood). The functional group used for the color development of Methotrexate was primary amine. The result obtaine in this method was based on coupling reaction formation reaction of Methotrexate with2, 2- Bipyridine / $Fecl_3$.

An attempt has been made to develop and validate to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in various gradients.

MATERIALS AND METHODS

(A). Preparation of Standard calibration curve of pure drug.

1. Solvent
Dimethyle Sulfoxide was used as Solvent.

2. Preparation of Calibration curve
Fresh aliquots of Methotrexate ranging from 0.4 to 2.4 (4 to24 µg ml^{-1}) were transferred into a series of 10ml volumetric flasks to provide final concentration range of 4 to24 µg ml^{-1}. To each flask 1 ml of (0.01M) 2, 2-Bipyridine solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to mark with Dimethyle Sulfoxide. The absorbance of orange red colored chromogen was measured at 510 nm against the reagent blank. The color species was stable for 24 h. The amount of Methotrexate present in the sample solution was computed from its calibration curve.

3. Procedure for formulations
Twenty tablets containing Methotrexate were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Methotrexate was dissolved in a 100 ml of Dimethyle Sulfoxide and mixed for about 5 min and then filtered. The Dimethyle Sulfoxide was evaporated to dryness. The remaining portion of solution was diluted in a 100ml volumetric flask to the volume with Dimethyle Sulfoxide up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100 ml volumetric flask and the volume was made up to the mark with Dimethyle Sulfoxide to obtain the final concentration of 100 µg ml^{-1} (Stock solution).

Subsequent dilutions of this solution were made with Dimethyle Sulfoxide to get concentration of 4 to24 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 510 nm and the results were statistically validated

4. Procedure for Blood sample

After collection of Blood sample it will be centrifuged. For isolation of Methotrexate from plasma sample, Dimethyle Sulfoxide was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M NaOH, followed by extraction with 30% dichloromethane in Hexane. The upper organic layer was evaporated to dryness, and the dry residue 100 mg was dissolved in 100 ml of Dimethyle Sulfoxide (1000 µg ml^{-1}). From the above solution 10 ml is taken into a 100 ml of Volumetric flask and made up to the mark with Dimethyle Sulfoxide .(100 µg ml^{-1})

From the above solution ranging from 0.4 to 2.4ml (4-24 µg ml^{-1}) were transferred in to 10 ml Volumetric flask and to the each flask 1ml of (0.01M) 2, 2- Bipyridine solution was added followed by 1ml of (0.2%) Ferric chloride solution and made up to the mark with Dimethyle Sulfoxide. Then the resulting solution was heated and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to the mark with Dimethyle Sulfoxide. The absorbance of orange red colored chromogen was measured at 510 nm against the reagent blank. The color species was stable for 24 h. The amount of Methotrexate present in the sample solution was computed from its calibration curve

RESULTS AND DISCUSSION

1. Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV spectrophotometric method and of the colored species formed in this visible spetrophotometric method, specified amount of Methotrexate in final solution 4-24 µg ml^{-1}(2, 2- Bipyridine Method) were taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 380-800 nm (2, 2- Bipyridine Method) against corresponding reagent blank. The regent blank absorption spectrum of each method was also recorded against distilled water / Dimethyle Sulfoxide. The results are graphically represented in (fig- 1).

2. Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development 2, 2- Bipyridine Method reproducibility and reasonable period of stability of final colored species formed. The following studies were conducted.

Method:

The results obtained in this method were based on oxidation followed by complex formation reaction of Methotrexate with 2,2-Bipyridine, Ferric chloride and Orthophosphoric acid to form an orange red colored chromogen that exhibited maximum absorption at 510 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of Methotrexate with 2, 2-Bipyridine reagent was shown in (fig-4). The effect of various parameters such as concentration and volume of 2,2-Bipyridine and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time.

3. Optical Characteristics

The reference method adhere to beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Methotrexate and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank.

The beers law plot of the system illustrated graphically (fig: 2) least square regression analysis was carried out for the slope. Intercept and Correlation Coefficient. Beer's law limits, Molar absorptivity & Sandells sensitivity for Methotrexate with each of mentioned reagents was calculated. The optical characteristics were present in the table-1.1.

Table-1.1: Optical characteristics and precision by (2, 2-B.P)

Parameter	Visible method
Absorption maxima (nm)	510
Beer's law limits (µg ml^{-1})	4-24
Molar absorptivity (l $mol^{-1}cm^{-1}$)	5.269×10^4
Sandell's Sensitivity (µg cm^{-2})	0.0452
Regression equation (Y*)	
Slope (b)	0.219
Intercept(a)	0.0016
Standard deviation(SD)	0.0008
Correlation coefficient (r^2)	0.9999
%RSD (Relative Standard deviation)	0.2859
Range of errors	
Confidence limits with 0.05 level	0.0006
Confidence limits with 0.01 level	0.0008
Limits of detection (LOD)(µg ml^{-1})	0.0109
Limits of quantification (LOQ) (µg ml^{-1})	0.0365

RSD of 6 independent determinations

Table-1.2: Assay results of Methotrexate in formulations by visible Method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method (mg)	% Recovery
FOLITRAX - 5	250	248.75 t=0.0029 F=8.5765	246.25	98.98
RHEUMATREX	250	248.00 t=0.0028 F=8.5678	245.5	98.99

- *t and F- values refer to comparison of the proposed method with reference method.*
- *Theoretical values at 95% confidence limits t= 0.00297 and F = 7.6177.*

Table-1.3: Determination of accuracy of Methotrexate

Amount of MET in Formulation (mg)	Amount of Standard MET added (mg)	Total amount found (mg)	% Recovery
248.33	200	446.99	99.33
247.91	200	446.23	99.16
247.70	200	445.86	99.08
248.25	250	496.5	99.3
248.33	250	496.66	99.33
248.83	250	497.66	99.53
248.33	300	546.32	99.33
248.47	300	546.63	99.32
248.60	300	546.92	99.44

Table-1.4: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
247.98	**0.3207**	**0.1293**
248.47	**0.3143**	**0.1264**
248.46	**0.1350**	**0.0543**

The results are the mean of three readings at each level of recovered

Table-1.5: Repeatability data for MET at 510 nm

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%) RSD
4	0.0875	0.0874	0.0873	0.0874	0.0001	0.1144
8	0.173	0.172	0.171	0.172	0.001	0.5813
12	0.262	0.265	0.264	0.263	0.0015	0.5703
16	0.350	0.351	0.353	0.351	0.0015	0.4273
20	0.439	0.438	0.436	0.437	0.0015	0.3432
24	0.526	0.525	0.524	0.525	0.001	0.1904

Average of six determinations

Table-1.6: Color stability data for 2, 2- Bipyridine Method

Conc. in (µg ml^{-1})	Time in Hours							
12	4	8	12	16	20	24	28	32
	0.262	0.262	0.263	0.263	0.263	0.264	0.184	0.149

Table-1.7: Assay results of Methotrexate in Blood sample

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method in (mg)	Amount found by the reference method (mg)	% of Recovery
FOLITRAX – 5	5	2.98 t=0.0029 F=0.0004	2.86	95.80
RHEUMATREX	5	2.97 t=0.0028 F=0.00039	2.88	96.87

- *t and F values refer to comparison of the proposed method with reference method.*
- *Theoretical values at 95% confidence limits t=0.00196 and F=0.00039.*

Table-1.8: Determination of accuracy of Methotrexate

The results are the mean of two readings at each level of recovered

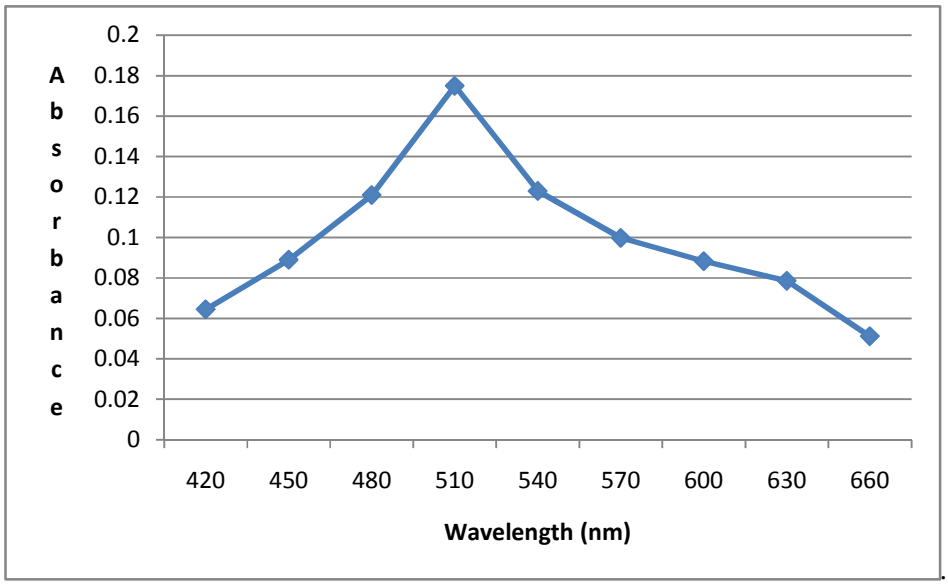
Name of the Formulation in (mg)	Amount of Drug in Blood sample (mg)	Amount of Standard Drug added in (mg)	Total amount found (mg)	% Recovery
5	2.97	10	8.91	59.40
5	2.99	10	8.97	59.80

Table-1.9: Repeatability data for Methotrexate at 510nm

Concentration in (µg ml^{-1})	Abs1	Abs2	Abs3	Mean	Std. Deviation	(%) RSD
0.5	0.066	0.065	0.067	0.066	0.0001	0.1515
1.0	0.132	0.131	0.139	0.134	0.0004	0.3208
1.5	0.198	0.197	0.197	0.197	0.0005	0.2538
2.0	0.264	0.265	0.267	0.265	0.0015	0.5660
2.5	0.33	0.321	0.324	0.325	0.0004	0.123
3.0	0.396	0.395	0.395	0.395	0.0005	0.1265

Average of six determinations

Fig-1: Absorption spectrum of Methotrexate with 2, 2-Bipyridine/$FeCl_3$



In order to test whether the colored species formed in the method adhere the beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Methotrexate and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The beers law plots of the system illustrated graphically (fig – 2&3) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandells sensitivity for Methotrexate with each of mentioned reagents were calculated. The optical characteristics are presented in the tables -1.1.

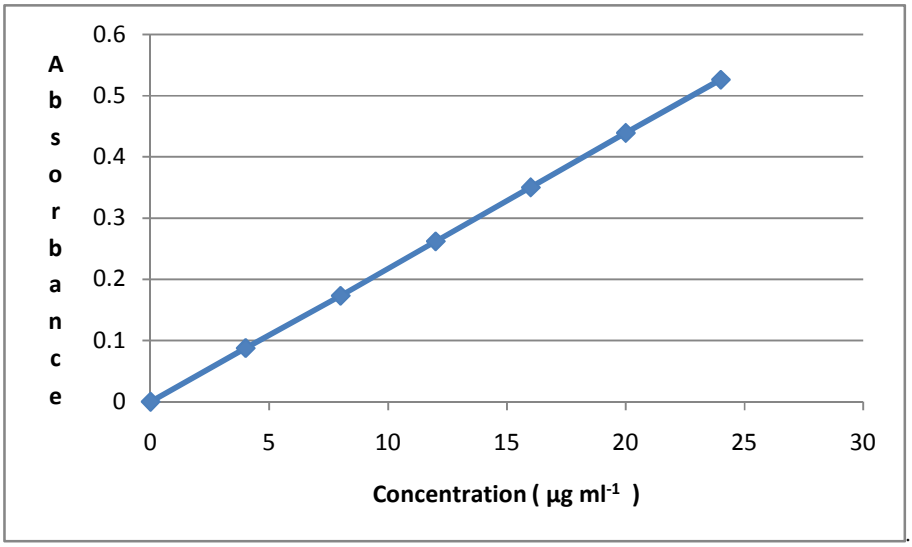
4. Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtain by actual determination of a fixed amount of Methotrexate in 4 µg ml^{-1} respectively - in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in table – 1.1.

5. Analysis of formulations

Commercial formulations of Methotrexate were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in table-1.2. The proposed methods also applied for Biological Samples (Blood) for good recoveries are obtained which were recorded in table-1.7.

Fig-2: Beer's law plot of Methotrexate with 2, 2-Bipyridine/$FeCl_3$



Accuracy

Recovery studies were carried by applying the Standared addition method to Drugs sample present in formulations for the known amount of Methotrexate the recovery studies were carried .By applying the same method to Biological sample (Blood) to which known amount of Methotrexate correspond to 2 mg Formulations taken by the patient. By the follow of Standard addition method 2 mg of label claim was added. After the addition of these standards the contents were transferred to 100 ml volumetric flash and dissolved in solvent. Finally the volume was made up to the mark with solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analyzed and their absorbance value was determined. At each level of recovery five determinations were performed and present in Table – 1.3. The results obtain were compared with expected results and were statistically validated in Table-1.4.

6. Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample with in a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

7. Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre weighed quantity of Drugs and then absorbance was measured and calculations were done to determine the quantity of the Drugs.

Fig.3: Beer's law plot for Methotrexate In Blood sample

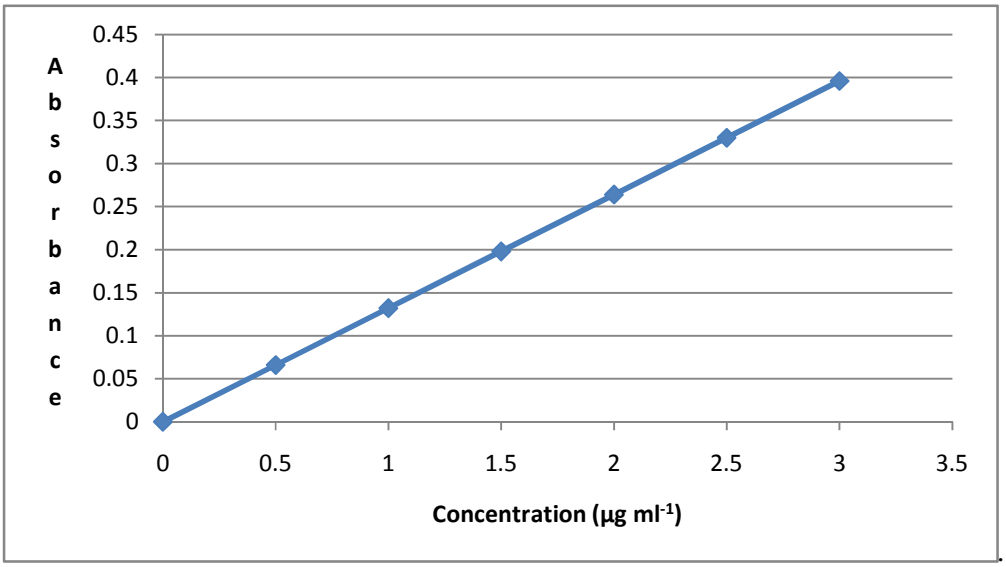
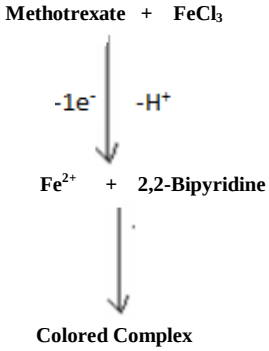


Fig.4: A Schematic reaction Mechanism of Methotrexate with 2, 2-Bipyridine



8. Repeatability

Standard solutions of Methotrexate were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure five times and standard deviation was calculated and presented in table -1.5&1.9.

9. Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Methotrexate under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in formulations.

REFERENCES

[1] K. Foulmann, J.P. Guastalla, N. Caminet, V. Trillet-Lenoir, D. Raudrant, F. Golfier, A.M. Schott, *Gynecologic Oncology* 102 (**2006**) 103–110.
[2] N.M. Annest, M.J. VanBeek, C.J. Arpey, D.C. Whitaker, *J. American Academy of Dermatology* 56 (**2007**) 989–993.
[3] T. Shingaki, D. Inoue, T. Furubayashi, T. Sakane, H. Katsumi, A. Yamamoto, S. Yamashita, *Molecular Pharmaceutics* 7 (**2010**)1561–1568.
[4] M. Dabrowska, M. Skoneczny, W. Rode, *Tumor Biology* 32 (**2011**) 965–976.
[5] R. Paliwal, S.R. Paliwal, N. Mishra, A. Mehta, S.P. Vyas, *International, Pharmaceutics*.380 (**2009**)181-188.
[6] M. Moniruzzaman, N. Kamiya, M. Goto, *J. Colloid and Interface Science* 352 (**2010**) 136–142.
[7] S. Natali, J. Mijovic, *Macromolecules* 43 (**2010**) 3011–3017
[8] L.M. Kaminskas, B.D. Kelly, V.M. McLeod, G. Sberna, B.J. Boyd, D.J. Owen, C.J.H. Porter, *Molecular Pharmaceutics* 8 (**2010**) 338–349.
[9] L. Ouyang, L.F. Ma, B. Jiang, Y.H. Li, D.S. He, L. Guo, *European J. Medicinal Chemistry* 45 (**2010**) 2705–2711.
[10] L.M. Kaminskas, B.D. Kelly, V.M. McLeod, B.J. Boyd, G.Y. Krippner, E.D. Williams, C.J.H. Porter, , *Molecular Pharmaceutics* 6 (**2009**) 1190–1204.
[11] R.S. Dhanikula, A. Argaw, J.F. Bouchard, P. Hildgen, *Molecular Pharmaceutics* (**2008**) 105–116.
[12] Y. Wang, X. Yang, J. Yang, Y. Wang, R. Chen, J. Wu, Y. Liu, N. Zhang, *Carbohydrate Polymers* 86 (**2011**) 1665–1670.
[13] M.R. Saboktakin, R.M. Tabatabaie, A. Maharramov, M.A. Ramazanov, *International Biological Macromolecules* 49 (**2011**) 747–751.
[14] Y.H. Chen, C.Y. Tsai, P.Y. Huang, M.Y. Chang, P.C. Cheng, C.H. Chou, D.H. Chen, C.R. Wang, A.L. Shiau, C.L. Wu, *Molecular Pharmaceutics* 4 (**2007**) 713–722.
[15] N. Kohler, C. Sun, J. Wang, M.Q. Zhang, *Langmuir* 21 (**2005**) 8858–8864.
[16] Y. Kawashima, *Advanced Drug Delivery Reviews* 47 (**2001**) 1–2.
[17] C. Chaumeil, *Methods and Findings in Experimental and Pharmacology20* (**1998**) 211–215.
[18] H.B. Chen, C. Khemtong, X.L. Yang, X.L. Chang, J.M. Gao, *Drug Discovery Today* 16 (**2011**) 354–360.
[19] C. Klingler, B.W. Mueller, H. Steckel, *International J. Pharmaceutics* 377 (**2009**) 173–179.
[20] A. Schoubben, P. Blasi, S. Giovagnoli, C. Rossi, M. Ricci, *Chemical Engineering* J. 160 (**2010**) 363–369.
[21] N. Rasenack, B.W. Muller, *Pharmaceutical Development and* 9 (**2004**) 1–13.
[22] D.M. Oh, R.L. Curl, C.S. Yong, G.L. Amidon, *Archives of Pharmacological Research* 18 (**1995**) 427–433.
[23] K.C. Johnson, A.C. Swindell, *Pharmaceutical* 13 (**1996**) 1795–1798.
[24] G.K. Reynolds, *Chemical Engineering J.* 164 (**2010**) 383–392.
[25] K.P. Hapgood, R. Amelia, M.B. Zaman, B.K. Merrett, P. Leslie, *Chemical Engineering J*. 164 (**2010**) 340–349.
[26] V.R. Nalluri, P. Schirg, X. Gao, A. Virdis, G. Imanidis, M. Kuentz,And *International J. Pharmaceutics* 391 (**2010**) 107–114.
[27] P. Balá ˇ z, A.V. Nguyen, M. Fabián, D. Cholujová, M. Pastorek, J. Sedlák, Z. Buj náková, *Powder Technology* 211 (**2011**) 232–236. 4
[28] A. Hassanpour, M. Ghadiri, A.C. Bentham, D.G. Papadopoulos, *Advanced Powder Technology* 14 (**2003**) 427–434.
[29] A. Hassanpour, M. Ghadiri, A.C. Bentham, D.G. Papadopoulos, *Powder Technology* 141 (**2004**) 239–243.
[30] L. Kuerti, A. Kukovecz, G. Kozma, R. Ambrus, M.A. Deli, P. Szabó-Révész, *Powder Technology* 212 (**2011**) 210–217.
[31] S. Garnier, S. Petit, F. Mallet, M.N. Petit, D. Lemarchand, S. Coste, J. Lefebvre,G, Coquerel, *International J. Pharmaceutics* 361 (**2008**)131–140.
[32] S. Park, S. Hwang, J. Lee, pH-responsive hydrogels from moldable composite microparticles prepared by coxial electro-spray drying on Chemical Engineering J. 169(**2011**)348-357.
[33] Y. He, Y.B. Huang, W.T. Wang, Y. Cheng, *Chemical Engineering J*. 168 (**2011**) 931-937.
[34] E. Reverchon, I. De Marco, *Chemical Engineering J*. 169 (**2011**) 358–370.

[35] E. Torino, I. De Marco, E. Reverchon, *J. Supercritical Fluids* 55 (**2010**) 300–306.
[36] E. Reverchon, E. Torino, S. Dowy, A. Braeuer, A. Leipertz, *Chemical Engineering J.* 156 (**2010**) 446–458.
[37] I. De Marco, E. Reverchon, *J. Supercritical Fluids* 58 (**2011**) 295–302.
[38] Y. Li, D.J. Yang, S.L. Chen, S.B. Chen, A.S.C. Chan, *International J. Pharmaceutics* 359 (**2008**) 35–45.
[39] A.Z. Chen, Y. Li, F.T. Chau, T.Y. Lau, J.Y. Hu, Z. Zhao, D.K.W. Mok, *J. Supercritical Fluids* 49 (**2009**) 394–402. 2
[40] Y.S. Youn, J.H. Oh, K.H. Ahn, M. Kim, J. Kim, Y.W. Lee, *J. Supercritical Fluids* 59 (**2011**)117–123.
[41] S.M. 2 Li, Y. Liu, T. Liu, L. Zhao, J.H. Zhao, N.P. Feng, *International J. Pharmaceutics* 411 (**2011**) 172–177.
[42] X. Zhao, Y. Zu, Q.Li,M, M. Wang, B.Zu, X.Zhang, R.Jiang, C. Zu, *J. Supercritical Fluids* 51 (**2010**) 412–419.
[43] A.Z. Chen, X.M. Pu, Y.Q. Kang, L. Liao, Y.D. Yao, G.F. Yin, *Macromolecular Rapid Communications* 27 (**2006**) 1254–1259. 2
[44] E. Reverchon, G. Della Porta, M.G. Falivene, *J. Supercritical Fluids* 17 (**2000**) 239–248.
[45] E. Reverchon, *J. Supercritical Fluids* 15 (**1999**) 1–21.
[46] M. Rantakylä, M. Jantti, O. Aaltonen, M. Hurme, *J. Supercritical Fluids* 24 (**2002**) 25–263.
[47] P.H.L. Tran, H.T.T. Tran, B.J. Lee, *J. Controlled Release* 129 (**2008**) 59–65.