RESEARCH ARTICLE

REVISED Machine learning meets pK_a [version 2; peer review: 2 approved]

Marcel Baltruschat, Paul Czodrowski

Faculty of Chemistry and Chemical Biology, TU Dortmund University, Otto-Hahn-Strasse 6, 44227 Dortmund, Germany

v2 **First published:** 13 Feb 2020, **9**(Chem Inf Sci):113
https://doi.org/10.12688/f1000research.22090.1

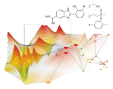
Latest published: 27 Apr 2020, **9**(Chem Inf Sci):113
https://doi.org/10.12688/f1000research.22090.2

Abstract

We present a small molecule pK_a prediction tool entirely written in Python. It predicts the macroscopic pK_a value and is trained on a literature compilation of monoprotic compounds. Different machine learning models were tested and random forest performed best given a five-fold cross-validation (mean absolute error=0.682, root mean squared error=1.032, correlation coefficient r^2 =0.82). We test our model on two external validation sets, where our model performs comparable to Marvin and is better than a recently published open source model. Our Python tool and all data is freely available at https://github.com/czodrowskilab/Machine-learning-meets-pKa.

Keywords

machine learning, pKa value, protonation, dissociation



This article is included in the Chemical Information Science gateway.

This article is included in the Python collection.

This article is included in the Mathematical, Physical, and Computational Sciences collection.

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 2 (revision) 27 Apr 2020		
version 1 13 Feb 2020	✓ report	✓ report

1 **Ruth Brenk**, University of Bergen, Bergen, Norway

2 **Johannes Kirchmair**, University of Vienna, Vienna, Austria

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Paul Czodrowski (paul.czodrowski@tu-dortmund.de)

Author roles: Baltruschat M: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Czodrowski P**: Conceptualization, Formal Analysis, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Baltruschat M and Czodrowski P. **Machine learning meets pK $_a$ [version 2; peer review: 2 approved]** F1000Research 2020, **9**(Chem Inf Sci):113 https://doi.org/10.12688/f1000research.22090.2

First published: 13 Feb 2020, **9**(Chem Inf Sci):113 https://doi.org/10.12688/f1000research.22090.1

REVISED **Amendments from Version 1**

We have made the following changes from version1 to version2:

- Figure 1 is now Figure 3 (requested by Reviewer1)
- Figure 2 is now Figure 4 (requested by Reviewer1)
- Figure2(A) and Figure 2(B) are new (requested by Reviewer2)
- Figure3 is now Figure1 (requested by Reviewer1)

We have transformed the text to the past tense (requested by Reviewer1). We also included text and Figure2A/Figure2B about the composition of the external Novartis test set.

As requested by Reviewer1: In the discussion section, the developed method are now discussed first before discussing its performance relative to other methods.

Any further responses from the reviewers can be found at the end of the article

Introduction

The acid-base dissociation constant (pK_a) of a drug has a far-reaching influence on pharmacokinetics by altering the solubility, membrane permeability and protein binding affinity of the drug. Several publications summarize these findings in a very comprehensive manner[1–7]. An accurate estimation of pK_a values is therefore of utmost importance for successful drug design. Several (commercial and non-commercial) tools and approaches for small molecule pK_a prediction are available: MoKa[8] uses molecular interaction fields, whereas ACD/Labs Percepta Classic[9], Marvin[10] and Epik[11] make use of the Hammet-Taft equation. By means of Jaguar[12], a quantum-mechanical approach to pK_a prediction becomes possible. Recently, the usage of neural nets for pK_a prediction became prominent[13–15]. In particular, the publication by Williams *et al.*[15] makes use of a publicly available data set provided by the application DataWarrior[16] and provides a freely available pK_a prediction tool called OPERA.

As this article is part of a Python collection issue, we provide a pK_a prediction method entirely written in Python[17] and make it available open source (including all data). Our tool computes the macroscopic pK_a value for a monoprotic compound. Our model solely differentiates between a base and acid based on the predicted pK_a value; i.e., we do not offer separate models for acids and bases. In addition to pK_a data from DataWarrior[16], we also employ pK_a data from ChEMBL[18]. As external validation sets, we use compound data provided by Novartis[19] and a manually curated data set compiled from literature[20–24], which are not part of the training data.

Methods

Data set preparation

A ChEMBL[18] web search was performed to find all assays containing pK_a measurement data. The following restrictions were made: it must be a physicochemical assay, the measurements must be taken from scientific literature, the assay must be in "small-molecule physicochemical format" and the organism taxonomy must be set to "N/A". This results in a list of 1140 ChEMBL assays downloaded as CSV file. Using a Python script, the CSV file was read in and processed further, extracting all additional information required from an internally hosted copy of the ChEMBL database via SQL. Only pK_a measurements, i.e. ChEMBL activities, were taken into account that were specified as exact ("standard_relation" equals "=") and for which one of the following names was specified as "standard_type": "pka", "pka value", "pka1", "pka2", "pka3" or "pka4" (case-insensitive). Measured values for which the molecular structure was not available were also sorted out. The resulting 8111 pK_a measured values were saved as SDF file.

A flat file from DataWarrior[16] named "pKaInWater.dwar" was used in addition. In this case, the file was converted to an SDF file only and contains 7911 entries with valid molecular structures.

These data sets were concatenated for the purpose of this study and preprocessed as follows:

- Removal of all salts from molecules
- Removal of molecules containing nitro groups, Boron, Selenium or Silicium
- Filtering by Lipinski's rule of five (one violation allowed)
- Keeping only pK_a data points between 2 and 12
- Tautomer standardization of all molecules
- Protonation of all molecules at pH 7.4
- Keeping only monoprotic molecules regarding the specified pK_a range
- Combination of data points from duplicated structures while removing outliers

All steps up to filtering out all pK_a values outside the range of 2 to 12 were performed with Python and RDKit[25]. The QuacPac[26] Tautomers tool from OpenEye was used for tautomer standardization and setting the protonation state to pH 7.4. The Marvin[10] tool from ChemAxon was used to filter out the multiprotic compounds. It predicted the pK_a values of all molecules in the range 2 to 12 and then retained only those molecules where Marvin did not predict more than one pK_a in that range.

The removal of the outliers is performed in two steps. First, before combining multiple measurements for the same molecules, all entries where the pK_a predicted by ChemAxon's Tool Marvin differs from the experimental value by more than four log units are removed. All molecules were then combined on the basis of the canonical isomeric SMILES. In the second step, when combining several measured values of a molecule, all those values that deviate from the mean value by more than two standard deviations are removed. The remaining values are arithmetically averaged.

After all, 5994 unique monoprotic molecules with experimental pK_a values remained. The distribution of pK_a values is given in Figure 1. The same preprocessing steps were also

performed on an external test data set provided to us by Novartis[19] (280 molecules) and a manual curation (123 molecules) from literature[20–24]. The Novartis data set consists of 280 unique molecules with a molecular weight between 129 and 670 daltons (mean value 348.68, standard deviation 94.17). The calculated LogP values vary between -1.54 and 6.30 (mean value 3.01, standard deviation 1.41). The 280 molecules spread over 228 unique Murcko Scaffolds. The ten most common murcko scaffolds cover 15% of the molecules of the total data set (42/280). A histogram of the pairwise comparison between the training set and the two external test sets (Fingerprint: 4096 bit MorganFeatures radius=3) is given in Figure 2(A) and Figure 2(B)

Learning

First, to simplify cross-validation, a class "CVRegressor" was defined, which can serve as a wrapper for any regressor implementing the Scikit-Learn[27] interface. This class simplifies cross-validation itself, training and prediction with the cross-validated model. Next, 196 of the 200 available RDKit descriptors ("MaxPartialCharge", "MinPartialCharge", "MaxAbsPartialCharge" and "MinAbsPartialCharge" were not used because they are computed as "NaN" for many molecules), and a 4096-bit long MorganFeature fingerprint with radius 3 were calculated for the training data set. Random forest (RF), support vector regression (SVR, two configurations), multilayer perceptron (MLP, three configurations) and XGradientBoost (XGB)

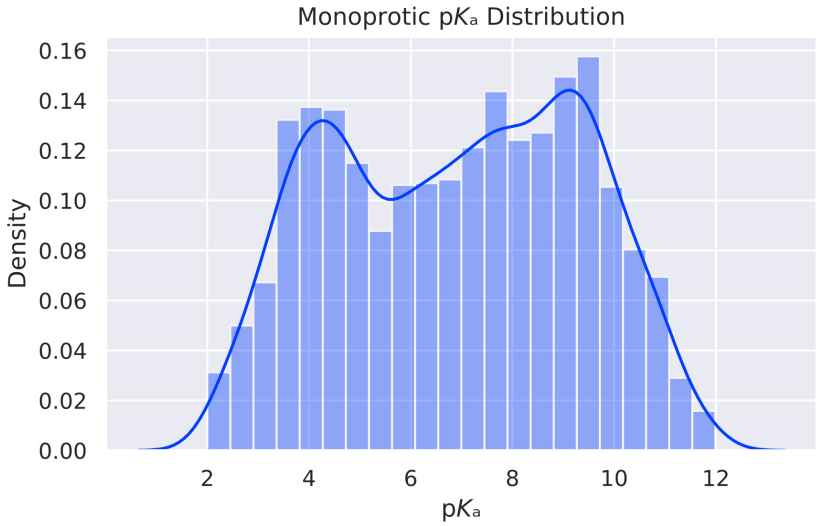


Figure 1. Distribution of the individual pK_a values.

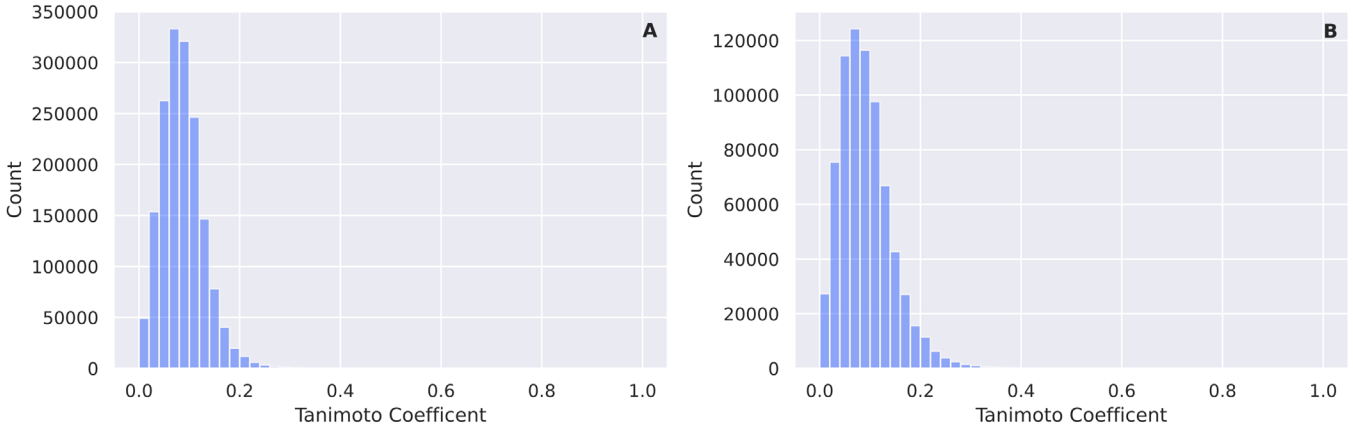


Figure 2. (**A**) Pairwise comparison between the training set and the Novartis test set (Fingerprint: 4096 bit MorganFeatures radius=3). (**B**) Pairwise comparison between the training set and the test set compiled by manual curation (Fingerprint: 4096 bit MorganFeatures radius=3).

were used as basic regressors. Unless otherwise specified, the Scikit-Learn default parameters (version 0.22.1) were used. For the RF model, only the number of trees was increased to 1000. For SVR the size of the cache was increased to 4096 megabytes in the first configuration, but this only increases the training speed and has no influence on the model quality. In the second configuration the parameter "gamma" was additionally set to the value "auto". For MLP in the first configuration the number of hidden layers was increased to two and the number of neurons per layer to 500. In the second configuration, early stopping was additionally activated, where 10% of the training data was separated as validation data. If the error of the validation data did not improve by more than 0.001 over ten training epochs, the training is stopped early to avoid overtraining. In the third configuration three hidden layers with a size of 250 neurons each were used with early stopping still activated. For XGB the default parameters of the used library XGBoost (version 0.90)[29] were applied. The training of RF, MLP and XGB was parallelized on 12 CPU cores and the generation of the folds for cross-validation as well as the training itself were random seeded to a value of 24 to ensure reproducibility. This resulted in a total of seven different machine learning configurations.

Six different descriptor/fingerprint combinations were also tested. First only the RDKit descriptors, followed by only the fingerprints and finally both combined. Additionally, all three combinations were tested again in a standardized form (z-transformed). As a result, 42 combinations of regressor and training data configurations were compared.

A 5-fold cross-validation was performed for all configurations, which were evaluated using the MAE, RMSE and the empirical coefficient of determination (r^2). After training was completed for all configurations, two external test data sets, which do not contain training data, were used to re-validate each trained cross-validated model. Here, MAE, RMSE, and r^2 were also calculated as statistical quality measures. To ensure that no training data was contained in the test data sets, the conical isomeric SMILES were checked for matches in both training and test data sets and corresponding hits were removed from the test data sets.

Implementation

The following Python dependencies have to be met: Python >= 3.7, NumPy >= 1.18, Scikit-Learn >= 0.22, RDKit >= 2019.09.3, Pandas >= 0.25, XGBoost >= 0.90, JupyterLab >= 1.2, Matplotlib >= 3.1, Seaborn >= 0.9

For the data preparation pipeline, ChemAxon Marvin[10] and OpenEye QUACPAC/Tautomers[26] are required. To use the provided prediction model with the included Python script, ChemAxon Marvin[10] is not required.

First of all a working Miniconda/Anaconda installation is needed. Miniconda can be downloaded at https://conda.io/en/latest/miniconda.html.

Now an environment named "ml_pka" with all needed dependencies can be created and activated with:

```
conda env create -f environment.yml
conda activate ml_pka
```

Alternatively, a new environment can be created manually without the environment.yml file:

```
conda create -n ml_pka python=3.7
conda activate ml_pka
```

In case of Linux or macOS:

```
conda install -c defaults -c rdkit -c conda-forge
  scikit-learn rdkit xgboost jupyterlab matplotlib
  seaborn
```

In case of Windows:

```
conda install -c defaults -c rdkit scikit-learn
  rdkit jupyterlab matplotlib seaborn pip install
  xgboost
```

Operation

Prediction pipeline. To use the data preparation pipeline the repository folder hast to be entered and the created conda environment must be activated. Additionally the Marvin[10] commandline tool `cxcalc` and the `QUACPAC`[26] commandline tool `tautomers` have to be added to the PATH variable.

Also the environment variables `OE_LICENSE` (containing the path to the OpenEye license file) and `JAVA_HOME` (referring to the Java installation folder, which is needed for `cxcalc`) have to be set.

After preparation a small usage information can be displayed with `bash run_pipeline.sh -h`. Examplary call:

```
bash run_pipeline.sh --train datasets/chembl25.
  sdf --test datasets/novartis_cleaned_mono_unique_
  notraindata.sdf
```

Prediction tool. First of all the repository folder has to be entered and the created conda environment must be activated. To use the prediction tool the machine learning model has to be retrained. To do so the training script should be called, it will train the 5-fold cross-validated Random Forest machine learning model using 12 cpu cores. If the number of cores has to be adjusted the `train_model.py` can be edited by changing the value of the variable `EST_JOBS`.

```
python train_model.py
```

To use the prediction tool with the trained model QUACPAC/Tautomers have to be available as mentioned in the section above.

Now the python script can be called with an SDF file and an output path:

```
python predict_sdf.py my_test_file.sdf my_output_
  file.sdf
```

It should be noted that this model was built for monoprotic structures regarding a pH range of 2 to 12. If the model is used with multiprotic structures, the predicted values will probably not be correct.

Results

Different experimental methods

One crucial point in the field of pK_a measurements (and its usage for pK_a predictions) was linked to the different experimental methods[25,30]. Based on the Novartis set, the correlation between capillary electrophoresis and potentiometric measurements (for 15 data points) was convincing enough (mean absolute error (MAE)=0.202, root mean squared error (RMSE)=0.264, correlation coefficient r^2=0.981) for us to combine pK_a measurements from these different experimental methods (see Figure 3).

We also compared the pK_a values of 187 monoprotic molecules contained in both the ChEMBL and DataWarrior data sets. Due to the missing annotation, it remained unclear if different experimental methods were used or multiple measurements with the same experimental method have been performed (or a mixture of both). Either way, this comparison was an additional proof-of-concept that the ChEMBL and DataWarrior pK_a data sources can be combined after careful curation. The aforementioned intersection is given in Figure 4. The correlation coefficient between the annotated pK_a values for these two data sets r^2 was 0.949, the MAE was 0.275, and the RMSE was 0.576.

The compounds for which the pK_a values between the different sources deviate by more than two units are as follows:

- Hydralazine:
  - pK_a (DataWarrior) 5.075
  - pK_a (ChEMBL25) 7.3
- Edaravone:
  - pK_a (DataWarrior) 11.3
  - pK_a (ChEMBL25) 6.9
- Trifluoromethanesulfonamide:
  - pK_a (DataWarrior) 6.33
  - pK_a (ChEMBL25) 9.7

Since the annotation about the experimental settings is not given in the DataWarrior file, we can only hypothesize that these differences are due to the different experimental settings.

Machine Learning

The statistics for a five-fold cross-validation are given in Table 1. In terms of the mean absolute error, a random forest with scaled MorganFeatures (radius=3) and descriptors gave the best performing model (MAE=0.682, RMSE=1.032, r^2=0.82). For the two external test sets (see Table 2), a random forest with FeatureMorgan (radius=3) gave the best model

- Novartis: MAE=1.147, RMSE=1.513, r^2=0.569
- LiteratureCompilation: MAE=0.532, RMSE=0.785, r^2=0.889)

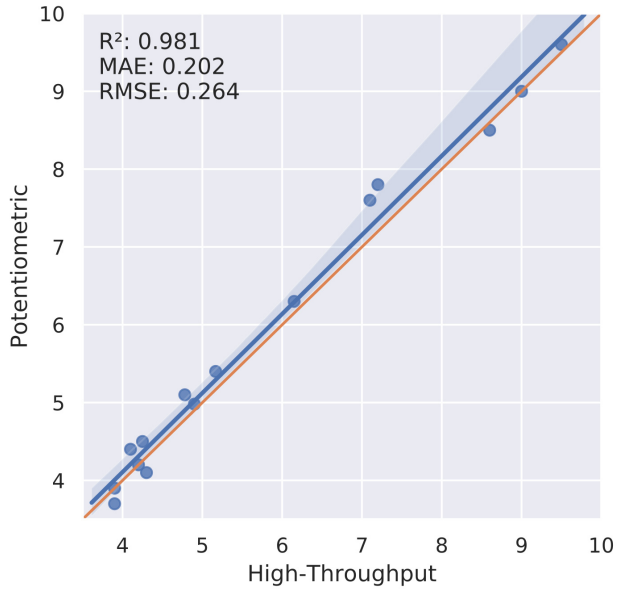


Figure 3. Correlation of Novartis compounds measured in potentiometric and high-throughput (capillary electrophoresis) set-up. MAE, mean absolute error; RMSE, root mean square error.

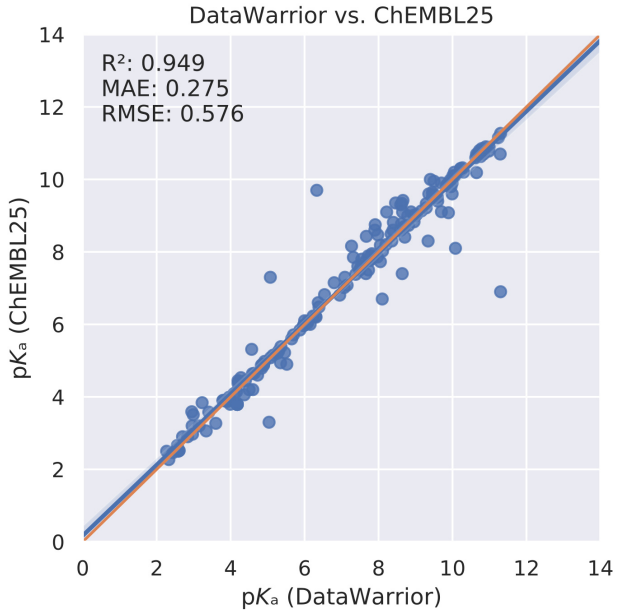


Figure 4. Intersection between ChEMBL and DataWarrior data sets. MAE, mean absolute error; RMSE, root mean square error.

Table 1. Statistics of the five-fold cross-validation of the machine learning models. The two best and worst performing models are highlighted in green and red. For those neural networks where the values were specified as "not available" ("#NA"), the weights could not be optimized properly due to the large value range of the RDKit descriptors, so training failed here.

		Cross-Validation					
Modell (seed=24)	**Train Configuration**	**MAE (mean)**	**MAE (std)**	**RMSE (mean)**	**RMSE (std)**	**R^2 (mean)**	**R^2 (std)**
Random Forest (n_estimators=1000)	**Desc (196 RDKit)**	0,718	0,022	1,077	0,021	0,804	0,01
	FCFP6 (4096 bit)	0,708	0,021	1,094	0,029	0,797	0,008
	Desc + FCFP6	0,683	0,017	1,032	0,013	0,82	0,005
	Desc (196 RDKit) (scaled)	0,717	0,022	1,076	0,022	0,804	0,011
	FCFP6 (4096 bit) (scaled)	0,708	0,021	1,094	0,029	0,797	0,008
	Desc + FCFP6 (scaled)	0,682	0,017	1,032	0,013	0,82	0,005
Support Vector Machine	**Desc (196 RDKit)**	2,1	0,037	2,436	0,035	-0,004	0,004
	FCFP6 (4096 bit)	0,851	0,025	1,24	0,035	0,74	0,012
	Desc + FCFP6	2,1	0,037	2,436	0,035	-0,004	0,004
	Desc (196 RDKit) (scaled)	0,876	0,033	1,282	0,047	0,722	0,015
	FCFP6 (4096 bit) (scaled)	1,09	0,034	1,466	0,041	0,637	0,014
	Desc + FCFP6 (scaled)	1,02	0,037	1,4	0,047	0,668	0,016
Support Vector Machine (gamma='auto')	**Desc (196 RDKit)**	2,016	0,042	2,362	0,039	0,056	0,009
	FCFP6 (4096 bit)	1,612	0,031	1,926	0,033	0,373	0,007
	Desc + FCFP6	1,642	0,061	2,052	0,06	0,288	0,027
	Desc (196 RDKit) (scaled)	0,882	0,035	1,288	0,048	0,719	0,016
	FCFP6 (4096 bit) (scaled)	1,09	0,034	1,465	0,041	0,637	0,014
	Desc + FCFP6 (scaled)	1,019	0,037	1,4	0,047	0,669	0,016
Multilayer Perceptron (hidden_layer_sizes=(500, 500))	**Desc (196 RDKit)**	#NA	#NA	#NA	#NA	#NA	#NA
	FCFP6 (4096 bit)	0,866	0,025	1,27	0,047	0,727	0,019
	Desc + FCFP6	#NA	#NA	#NA	#NA	#NA	#NA
	Desc (196 RDKit) (scaled)	0,726	0,018	1,102	0,05	0,794	0,022
	FCFP6 (4096 bit) (scaled)	1,037	0,045	1,457	0,057	0,64	0,024
	Desc + FCFP6 (scaled)	0,968	0,032	1,383	0,04	0,677	0,014
Multilayer Perceptron (hidden_layer_sizes=(500, 500), early_stopping=True)	**Desc (196 RDKit)**	#NA	#NA	#NA	#NA	#NA	#NA
	FCFP6 (4096 bit)	0,894	0,024	1,297	0,04	0,715	0,016
	Desc + FCFP6	#NA	#NA	#NA	#NA	#NA	#NA
	Desc (196 RDKit) (scaled)	0,768	0,034	1,161	0,09	0,77	0,038
	FCFP6 (4096 bit) (scaled)	1,031	0,037	1,447	0,057	0,645	0,026
	Desc + FCFP6 (scaled)	0,984	0,029	1,404	0,035	0,666	0,017
Multilayer Perceptron (hidden_layer_sizes=(250, 250, 250), early_stopping=True)	**Desc (196 RDKit)**	#NA	#NA	#NA	#NA	#NA	#NA
	FCFP6 (4096 bit)	0,869	0,023	1,265	0,039	0,729	0,016
	Desc + FCFP6	#NA	#NA	#NA	#NA	#NA	#NA
	Desc (196 RDKit) (scaled)	0,775	0,008	1,158	0,033	0,773	0,013
	FCFP6 (4096 bit) (scaled)	1,026	0,038	1,455	0,053	0,642	0,022
	Desc + FCFP6 (scaled)	0,973	0,035	1,388	0,053	0,674	0,023
XGBoost	**Desc (196 RDKit)**	1,02	0,014	1,353	0,021	0,691	0,007
	FCFP6 (4096 bit)	1,094	0,027	1,423	0,036	0,657	0,011
	Desc + FCFP6	1,018	0,01	1,346	0,022	0,694	0,005
	Desc (196 RDKit) (scaled)	1,02	0,014	1,353	0,021	0,691	0,007
	FCFP6 (4096 bit) (scaled)	1,094	0,027	1,423	0,036	0,657	0,011
	Desc + FCFP6 (scaled)	1,018	0,01	1,346	0,022	0,694	0,005

MAE, mean absolute error; RMSE, root mean square error.

Table 2. Predictive performance of the machine learning models the on the two external test sets. The two best and worst performing models are highlighted in green and red. For those neural networks where the values were specified as "not available" ("#NA"), the weights could not be optimized properly due to the large value range of the RDKit descriptors, so training failed here.

		Novartis			AvLiLuMoVe		
Modell (seed=24)	**Train Configuration**	**MAE**	**RMSE**	**R^2**	**MAE**	**RMSE**	**R^2**
Random Forest (n_estimators=1000)	**Desc (196 RDKit)**	1,259	1,607	0,513	0,689	0,979	0,828
	FCFP6 (4096 bit)	1,147	1,513	0,569	0,532	0,785	0,889
	Desc + FCFP6	1,2	1,532	0,558	0,628	0,884	0,86
	Desc (196 RDKit) (scaled)	1,259	1,607	0,513	0,688	0,979	0,828
	FCFP6 (4096 bit) (scaled)	1,147	1,513	0,569	0,532	0,785	0,889
	Desc + FCFP6 (scaled)	1,198	1,531	0,558	0,628	0,884	0,86
Support Vector Machine	**Desc (196 RDKit)**	2,177	2,451	-0,132	2,18	2,441	-0,07
	FCFP6 (4096 bit)	1,423	1,732	0,435	0,688	0,981	0,827
	Desc + FCFP6	2,177	2,451	-0,132	2,18	2,441	-0,07
	Desc (196 RDKit) (scaled)	1,382	1,735	0,433	0,772	1,058	0,799
	FCFP6 (4096 bit) (scaled)	1,771	2,035	0,219	1,115	1,422	0,637
	Desc + FCFP6 (scaled)	1,746	2,015	0,235	1,044	1,345	0,675
Support Vector Machine (gamma='auto')	**Desc (196 RDKit)**	2,162	2,428	-0,111	1,921	2,242	0,097
	FCFP6 (4096 bit)	1,686	1,932	0,297	1,429	1,67	0,499
	Desc + FCFP6	2,161	2,442	-0,124	1,611	2,004	0,279
	Desc (196 RDKit) (scaled)	1,378	1,732	0,435	0,766	1,049	0,802
	FCFP6 (4096 bit) (scaled)	1,77	2,034	0,22	1,114	1,421	0,637
	Desc + FCFP6 (scaled)	1,744	2,013	0,236	1,043	1,343	0,676
Multilayer Perceptron (hidden_layer_sizes=(500, 500))	**Desc (196 RDKit)**	#NV	#NV	#NV	#NV	#NV	#NV
	FCFP6 (4096 bit)	1,414	1,773	0,407	0,852	1,169	0,755
	Desc + FCFP6	#NV	#NV	#NV	#NV	#NV	#NV
	Desc (196 RDKit) (scaled)	1,318	1,634	0,497	0,688	0,942	0,841
	FCFP6 (4096 bit) (scaled)	1,627	2,033	0,221	1,102	1,569	0,558
	Desc + FCFP6 (scaled)	1,542	1,941	0,29	1,001	1,427	0,634
Multilayer Perceptron (hidden_layer_sizes=(500, 500), early_stopping=True)	**Desc (196 RDKit)**	#NV	#NV	#NV	#NV	#NV	#NV
	FCFP6 (4096 bit)	1,404	1,772	0,408	0,846	1,154	0,761
	Desc + FCFP6	#NV	#NV	#NV	#NV	#NV	#NV
	Desc (196 RDKit) (scaled)	1,298	1,626	0,502	0,701	0,936	0,843
	FCFP6 (4096 bit) (scaled)	1,611	2,028	0,225	1,141	1,575	0,554
	Desc + FCFP6 (scaled)	1,605	1,998	0,248	0,987	1,365	0,665
Multilayer Perceptron (hidden_layer_sizes=(250, 250, 250), early_stopping=True)	**Desc (196 RDKit)**	#NV	#NV	#NV	#NV	#NV	#NV
	FCFP6 (4096 bit)	1,363	1,717	0,445	0,86	1,164	0,757
	Desc + FCFP6	#NV	#NV	#NV	#NV	#NV	#NV
	Desc (196 RDKit) (scaled)	1,354	1,705	0,452	0,777	1,057	0,799
	FCFP6 (4096 bit) (scaled)	1,584	1,989	0,254	1,053	1,468	0,613
	Desc + FCFP6 (scaled)	1,581	1,963	0,274	0,953	1,352	0,672
XGBoost	**Desc (196 RDKit)**	1,367	0,453	1,704	0,819	0,806	1,04
	FCFP6 (4096 bit)	1,28	0,503	1,624	0,782	0,823	0,992
	Desc + FCFP6	1,293	0,495	1,637	0,774	0,822	0,995
	Desc (196 RDKit) (scaled)	1,367	0,453	1,704	0,819	0,806	1,04
	FCFP6 (4096 bit) (scaled)	1,28	0,503	1,624	0,782	0,823	0,992
	Desc + FCFP6 (scaled)	1,293	0,495	1,637	0,774	0,822	0,995
ChemAxon Marvin (V20.1.0)		0,856	1,166	0,744	0,566	0,865	0,866
OPERA (V2.5)*		2,274	3,059	-0,754	1,737	2,182	0,124

*For OPERA 6 molecules from AvLiLuMoVe and 31 molecules from Novartis were left out because OPERA predicted either two or zero pK_a values.
MAE, mean absolute error; RMSE, root mean square error.

The predictive performance for Marvin[10] and the OPERA tool[15] were as follows:

- Novartis
  - Marvin: MAE=0.856, RMSE=1.166, r^2=0.744
  - OPERA: MAE= 2.274, RMSE= 3.059, r^2= -0.754
- LiteratureComplation[20–24]
  - Marvin: MAE= 0.566, RMSE= 0.865, r^2= 0.866
  - OPERA: MAE= 1.737, RMSE= 2.182, r^2= 0.124.

This showed that our model had a slightly better performance than Marvin for the LiteratureCompilation, but Marvin performed better for the Novartis dataset. For both data sets, our models[17] had a better predictive performance than the OPERA tool. Since some molecules had to be omitted for prediction with OPERA due to none or multiple predicted pK_a values, no consistent significance test could be performed for all comparisons.

Discussion and conclusions

The developed model offers the possibility to predict pK_a values for monoprotic molecules with good accuracy. However, since the model has been trained exclusively with monoprotic molecules, only monoprotic molecules can be predicted properly. In this respect the model is limited. Nevertheless, the results show that the performance for monoprotic molecules can compete with the performance of existing prediction tools. The good performance of Marvin on the Novartis set is interesting to note: the RMSE was almost 0.4 units better than our top performing model. This could be because Marvin's training set is much larger than our own training set. This provides a better foundation for the training of the Marvin model. In contrast, Marvin performed slightly worse than our top model on the LiteratureCompilation. The OPERA tool performed significantly worse than our model on both external test sets. We assume that the addition of 2470 ChEMBL pK_a – datapoints to our training set which were not part of the OPERA training set led to this drop in predictive performance. In addition, the pre-processing of the data was performed differently by OPERA in comparison to our pre-processing procedure.

As next step for the enhancement and improvement of our pK_a prediction model[17], we are currently expanding it to multiprotic molecules. We are also investigating the impact of different neural net architectures and types (such as graph neural nets) and the development of individual models for acids and bases. From a chemistry perspective, an analysis of pK_a effects of different functional groups (e.g. by means of matched molecular pairs analysis) is an on-going effort for a future publication.

Data availability

Source data

Zenodo: czodrowskilab/Machine-learning-meets-pKa article. https://doi.org/10.5281/zenodo.3662245[17].

The following data sets were used in this study:

- `AvLiLuMoVe.sdf` - Manually combined literature pK_a data.
- `chembl25.sdf` - Experimental pK_a data extracted from ChEMBL25.
- `datawarrior.sdf` - pK_a data shipped with DataWarrior.
- `combined_training_datasets_unique.sdf` - Preprocessed and combined data from datasets chembl25.sdf and datawarrior.sdf, used as training dataset.
- `AvLiLuMoVe_cleaned_mono_unique_notraindata.sdf` - used as external testset.
- `novartis_cleaned_mono_unique_notraindata.sdf` - inhouse dataset provided by Novartis[19], used as external testset.

The data sets are also available at https://github.com/czodrowskilab/Machine-learning-meets-pKa.

License: MIT license.

Software availability

The source code is available at https://github.com/czodrowskilab/Machine-learning-meets-pKa.

Archived source code at time of publication: https://doi.org/10.5281/zenodo.3662245[17].

License: MIT license.

Acknowledgments

Ed Griffen (MedChemica) is acknowledged for his investigations on our initial data set which revealed some wrongly annotated data points. We thank Bob Clark, Eric Jamois and Michael Lawless (Simulations Plus) for their support and advise in terms of data curation. Alpha Lee and Matt Robinson (Cambridge University) are appreciated for fruitful discussions.

References

1. Manallack DT: **The p*K*$_a$ Distribution of Drugs: Application to Drug Discovery.** *Perspect Medicin Chem.* 2007; **1**: 25–38.
PubMed Abstract | Publisher Full Text | Free Full Text
2. Manallack DT, Yuriev E, Chalmers DK: **The influence and manipulation of acid/base properties in drug discovery.** *Drug Discov Today Technol.* 2018; **27**: 41–47.
PubMed Abstract | Publisher Full Text
3. Manallack DT, Prankerd RJ, Yuriev E, *et al.*: **The significance of acid/base properties in drug discovery.** *Chem Soc Rev.* 2013; **42**(2): 485–496.
PubMed Abstract | Publisher Full Text | Free Full Text
4. Meanwell NA: **Improving drug candidates by design: a focus on physicochemical properties as a means of improving compound disposition and safety.** *Chem Res Toxicol.* 2011; **24**(9): 1420–1456.
PubMed Abstract | Publisher Full Text
5. Gleeson MP: **Generation of a set of simple, interpretable ADMET rules of**

thumb. *J Med Chem.* 2008; **51**(4): 817–834.
PubMed Abstract | Publisher Full Text

6. Leeson PD, St-Gallay SA, Wenlock MC: **Impact of Ion Class and Time on Oral Drug Molecular Properties.** *Med Chem Commun.* 2011; **2**(2): 91–105.
Publisher Full Text

7. Charifson PS, Walters WP: **Acidic and basic drugs in medicinal chemistry: a perspective.** *J Med Chem.* 2014; **57**(23): 9701–9717.
PubMed Abstract | Publisher Full Text

8. Milletti F, Storchi L, Sforna G, *et al.*: **New and original pK_a prediction method using grid molecular interaction fields.** *J Chem Inf Model.* 2007; **47**(6): 2172–2181.
PubMed Abstract | Publisher Full Text

9. **ACD/Percepta, Advanced Chemistry Development, Inc**. Toronto, On Canada, Www.Acdlabs.Com, 2019.
Reference Source

10. **Marvin 20.1.0,** 2020. ChemAxon.
Reference Source

11. Shelley JC, Cholleti A, Frye LL, *et al.*: **Epik: a software program for pK_a prediction and protonation state generation for drug-like molecules.** *J Comput Aided Mol Des.* 2007; **21**(12): 681–691.
PubMed Abstract | Publisher Full Text

12. Bochevarov AD, Watson MA, Greenwood JR, *et al.*: **Multiconformation, Density Functional Theory-Based pK_a Prediction in Application to Large, Flexible Organic Molecules with Diverse Functional Groups.** *J Chem Theory Comput.* 2016; **12**(12): 6001–6019.
PubMed Abstract | Publisher Full Text

13. Fraczkiewicz R, Lobell M, Göller AH, *et al.*: **Best of both worlds: combining pharma data and state of the art modeling technology to improve *in Silico* pK_a prediction.** *J Chem Inf Model.* 2015; **55**(2): 389–397.
PubMed Abstract | Publisher Full Text

14. Roszak R, Beker W, Molga K, *et al.*: **Rapid and Accurate Prediction of pK_a Values of C-H Acids Using Graph Convolutional Neural Networks.** *J Am Chem Soc.* 2019; **141**(43): 17142–17149.
PubMed Abstract | Publisher Full Text

15. Mansouri K, Cariello NF, Korotcov A, *et al.*: **Open-Source QSAR Models for PKa Prediction Using Multiple Machine Learning Approaches.** *J Cheminform.* 2019; **11**(1): 60.
Publisher Full Text | Free Full Text

16. Sander T, Freyss J, von Korff M, *et al.*: **DataWarrior: an open-source program for chemistry aware data visualization and analysis.** *J Chem Inf Model.* 2015; **55**(2): 460–473.
PubMed Abstract | Publisher Full Text

17. Lewis RA, Rodde S: **Novartis Pharma AG.** Basel, Switzerland.

18. Settimo L, Bellman K, Knegtel RM: **Comparison of the accuracy of experimental and predicted pKa values of basic and acidic compounds.** *Pharm Res.* 2014; **31**(4): 1082–1095.
PubMed Abstract | Publisher Full Text

19. Liao C, Nicklaus MC: **Comparison of nine programs predicting pK_a values of pharmaceutical substances.** *J Chem Inf Model.* 2009; **49**(12): 2801–2812.
PubMed Abstract | Publisher Full Text

20. Avdeef A: **Absorption and Drug Development: Solubility, Permeability, and Charge State**. John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2012.
Publisher Full Text

21. Morgenthaler M, Schweizer E, Hoffmann-Röder A, *et al.*: **Predicting and tuning physicochemical properties in lead optimization: amine basicities.** *ChemMedChem.* 2007; **2**(8): 1100–1115.
PubMed Abstract | Publisher Full Text

22. Luan F, Ma W, Zhang H, *et al.*: **Prediction of pK_a for neutral and basic drugs based on radial basis function Neural networks and the heuristic method.** *Pharm Res.* 2005; **22**(9): 1454–1460.
PubMed Abstract | Publisher Full Text

23. Dardonville C: **Automated techniques in pK_a determination: low medium and high-throughput screening methods.** *Drug Discov Today Technol.* 2018; **27**: 49–58.
PubMed Abstract | Publisher Full Text

24. Reijenga J, van Hoof A, van Loon A, *et al.*: **Development of Methods for the Determination of pK_a Values.** *Anal Chem Insights.* 2013; **8**(1): 53–71.
PubMed Abstract | Publisher Full Text | Free Full Text

25. **RDKit, Open-Source Chemiformatics**.
Reference Source

26. QUACPAC 2.0.2.2: **OpenEye Scientific Software.** Santa Fe, NM.
Reference Source

27. Pedregosa F, Weiss R, Brucher M: **Scikit-Learn: Machine Learning in Python.** *J Mach Learn Res.* 2011; **12**: 2825–2830.
Reference Source

28. Chen T, Guestrin C: **XGBoost: A Scalable Tree Boosting System**. In *Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining - KDD '16*; ACM Press: New York, New York, USA. 2016; 785–794.
Publisher Full Text

29. Baltruschat M, czodrowskilab, Czodrowski P: **czodrowskilab/Machine-learning-meets-pKa article (Version article).** *Zenodo.* 2020.
http://www.doi.org/10.5281/zenodo.3662245

30. Gaulton A, Hersey A, Nowotka M, *et al.*: **The ChEMBL database in 2017.** *Nucleic Acids Res.* 2017; **45**(D1): D945–D954.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 24 March 2020

https://doi.org/10.5256/f1000research.24362.r61511

✔ **Johannes Kirchmair**
Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Vienna, Austria

Baltruschat and Czodrowski report on the development of a Python-based tool for the prediction of pk_a values. The tool is made available open source and will certainly be useful to the scientific community.

- Could the authors please comment on the structural relationship between the training and the test data (how far apart are these datasets, e.g. measured by the distribution of pairwise Tanimoto coefficients based on molecular fingerprints)?
- Could the authors please confirm statistical significance of the key statements made in the first paragraph of the Results section, starting with "This shows that our model slightly outcompetes..."?
- In the Abstract, please name the open source model that was tested as part of the comparative performance analysis.
- It is not clear from the manuscript text whether capillary electrophoresis and potentiometric measurements were the only two experimental methods considered in this work (or, for the Novartis dataset only).
- Please revise the sentence starting with "We also compare the overlap...".
- "make use of a" should be replaced by "makes use of a".
- Please explain where DataWarrior's data originates from.
- Please comment on the outliers observed in Figure 2.
- Please explain the procedure that was followed to ensure that there was no overlap between the training data and the external validation sets (the Novartis dataset in particular).
- "isomeric SMILES": is it canonical as well?

- Avoid using "you" in the Implementation section (use "one [can]..." instead).
- The part starting with "For the two external test sets..." is difficult to read. Could the authors find a more simple way to describe this and enable readers to compare the individual values more easily?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cheminformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 15 Apr 2020

Paul Czodrowski, TU Dortmund University, Otto-Hahn-Strasse 6, Germany

Could the authors please comment on the structural relationship between the training and the test data (how far apart are these datasets, e.g. measured by the distribution of pairwise Tanimoto coefficients based on molecular fingerprints)?
We have included new figures (2A and 2B) with pairwise Tanimoto coefficients (4096 bit MorganFeatures radius=3).

Could the authors please confirm statistical significance of the key statements made in the first paragraph of the Results section, starting with "This shows that our model slightly outcompetes..."?
A thorough test of the statistical significance is hindered by the fact the external open source tool OPERA cannot process all molecules of our external test sets. We therefore decided to rephrase "This showed that our model slightly outcompeted Marvin for the LiteratureCompilation" to "his showed that our model has a slightly better performance than Marvin for the LiteratureCompilation".

In the Abstract, please name the open source model that was tested as part of the comparative

performance analysis.
The open source model is now mentioned in the abstract.

It is not clear from the manuscript text whether capillary electrophoresis and potentiometric measurements were the only two experimental methods considered in this work (or, for the Novartis dataset only).
We mention this already in the section “Different experimental methods” :“Due to the missing annotation, it remained unclear if different experimental methods were used or multiple measurements with the same experimental method have been performed (or a mixture of both)”.

Please revise the sentence starting with "We also compare the overlap...".
Changed "We also compared the overlap of the filtered (see next section) ChEMBL and DataWarrior data sets, 187 monoprotic molecules could be identified in both sources." To "We also compared the pKa values of 187 monoprotic molecules contained in both the ChEMBL and DataWarrior data sets."

"make use of a" should be replaced by "makes use of a".
Done

Please explain where DataWarrior's data originates from.
We cannot make any statement about the origin, because it is not included in the DataWarrior file.

Please comment on the outliers observed in Figure 2.
We include the name of the outliers and the different pKa values originating from different data sources, if they differ by more than two log units.

Please explain the procedure that was followed to ensure that there was no overlap between the training data and the external validation sets (the Novartis dataset in particular).
To ensure that no training data was contained in the test data sets, the canonical isomeric SMILES were checked for matches in both training and test data sets and corresponding hits were removed from the test data sets. Chirality was not included in this comparison.

"isomeric SMILES": is it canonical as well?
Yes, it is the canonical isomeric SMILES generated by RDKit.

Avoid using "you" in the Implementation section (use "one [can]..." instead).
Done

The part starting with "For the two external test sets..." is difficult to read. Could the authors find a more simple way to describe this and enable readers to compare the individual values more easily?
We have pulled most of the numbers apart and included bullet points which improves the readability and makes a comparison more feasible.

Competing Interests: No competing interests were disclosed.

Reviewer Report 25 February 2020

https://doi.org/10.5256/f1000research.24362.r60011

Ruth Brenk
Department of Biomedicine, University of Bergen, Bergen, Norway

The article describes a machine learning method for pkA prediction. The presented method and results are scientifically solid. Therefore, I recommend the article for indexing. However, the methods and results should be presented more clearly before the article is indexed.

The following points should be addressed:

1. Past tense should be used consistently to describe the methods and results.

2. The “different experimental methods” should be moved to the results part as it contains results.

3. A short description of the molecules in the self-compiled data set and the Novartis set should be added. What type of molecules are contained in these sets?

4. Why does the user need to train the model before making predictions? Could the trained model be provided to make the developed tool easier to use?

5. In the discussion section, the developed method should be first discussed before discussing its performance relative to other methods.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: chemoinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 05 Mar 2020

Paul Czodrowski, TU Dortmund University, Otto-Hahn-Strasse 6, Germany

1. Past tense should be used consistently to describe the methods and results.*We have used past tense throughout the entire document.*
2. The “different experimental methods” should be moved to the results part as it contains results.*This part was moved accordingly.*
3. A short description of the molecules in the self-compiled data set and the Novartis set should be added. What type of molecules are contained in these sets?*We have added this information in the manuscript: "The Novartis data set consists of 280 unique molecules with a molecular weight between 129 and 670 daltons (mean value 348.68, standard deviation 94.17). The calculated LogP values vary between -1.54 and 6.30 (mean value 3.01, standard deviation 1.41). The 280 molecules spread over 228 unique Murcko Scaffolds. The ten most common murcko scaffolds cover 15% of the molecules of the total data set (42/280)."*
4. Why does the user need to train the model before making predictions? Could the trained model be provided to make the developed tool easier to use?*The user has to train the model again by himself or herself, because the model is larger than 1.7 GB as a file and therefore cannot be offered for download without paid services. Even with the best possible bzip2 compression, the file is still over 250 MB in size and thus larger than the 100 MB limit at GitHub. In addition, if the model is provided as a file, you must use exactly the same versions of the Python libraries used for training to load the model, otherwise inconsistencies may occur. However, if the model is trained again by yourself, only the minimum library requirements have to be met.*
5. In the discussion section, the developed method should be first discussed before discussing its performance relative to other methods.*This was done and is reflected in the new manuscript version.*

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Author Response 05 Mar 2020

Paul Czodrowski, TU Dortmund University, Otto-Hahn-Strasse 6, Germany

Thanks for the comments, we will take a closer look at the distinction between acidic and basic pKa and the different OPERA models in a follow-up publication.

Currently, a check of the molecules to be predicted with respect to the applicability of the trained model is

not implemented, because otherwise the Marvin tool from ChemAxon would also be required for the prediction. With Marvin the classification between mono- and multiprotic molecules is performed. However, we are considering adding an optional validation option for the input structures.

Competing Interests: No competing interests were disclosed.

Reader Comment 20 Feb 2020

Kamel Mansouri, ILS, USA

This is a good attempt to model pKa which is an important parameter to predict. This type of work is also a good addition to the existing free and open-source pKa predictors such as OPERA.
The authors clearly state that their tool only works on monoprotic chemicals and doesn't differentiate between acidic and basic pKa. However, in their comparison with other tools that do, the authors fail to make that differentiation. To compare the comparable, the authors should compare their predictions with OPERA predictions separately for acidic and basic pKa also because one of their data sources was DataWarrior (also used for OPERA training and testing) differentiates between the two (acidic Vs basic).

Since the model only works for monoprotic chemicals, is there an applicability domain assessment or some sort of error message that informs the user when they try to use for a multiprotic chemical? if not, it should be added.

Both the results and discussion sections should expanded further and clarify the strengths and limitations of the tool.

Competing Interests: I have no competing interests.